PLEON
POCONO LAKE ECOLOGICAL OBSERVATORY NETWORK

To the Twin and Walker Creeks Watershed Conservancy

Report of 2023 PLEON Sampling

From the Pocono Lakes Ecological Observatory Network

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I. Summary 2023: Twin and Walker Lakes at a Glance

PLEON partnered with TWCWC to monitor Big Twin, Little Twin, and Walker lakes three times in 2023 with PLEON on-site in July. Big Twin samples was screened for potentially toxigenic cyanobacteria once.

Table 1: Summary of 2023 monitoring.

	Variables Monitored	**Crew**
17 June 18 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) • Dissolved organic carbon (0.5 m)	Collection: TWCWC Analysis: PLEON
15 July* 17 July	• Profiles: temperature, dissolved oxygen, conductivity, pH, light • Secchi Depth • Chlorophyll a (0.5 m*, composite*, hypolimnion (Twin lakes only)) • Total N, Total P (0.5 m*, composite*, hypolimnion (Twin lakes only) • Dissolved organic carbon (0.5 m)* • Zooplankton and phytoplankton community (Twin Lakes only)	Sample Collection: TWCWC Alexandra Bros (PLEON Technician) Brayden Furnas (Intern) Huey James (Intern)
19 Aug 20 Aug	• Profiles: temperature, dissolved oxygen, conductivity, pH • Secchi Depth • Chlorophyll a (0.5 m, composite, hypolimnion (Twin lakes only)) • Total N, Total P (0.5 m, composite, hypolimnion (Twin lakes only)) • dissolved organic carbon (0.5 m)	Collection: TWCWC Analysis: PLEON
21 Aug	• PTOX screens (Big Twin)	Collection: TWCWC Analysis: PLEON (Greenwater Labs)

Table 2: Summary of Big Twin Lake in 2023

	17 June	**17 July****	**19 Aug**	**21 Aug**
Thermally stratified?	YES	YES	YES	—
Epilimnion depth (m)	4	3	5	—
Metalimnion depth (m)	7	8	8	—
Secchi depth (m)	2.6	2	2.3	—
Vertical extinction coefficient (k)	—	1.14	—	—
$Z_{10\%}$ (m)	—	2.03	—	—
$Z_{1\%}$ (m)	—	4.06	—	—
Mean hypolimnetic DO (mg/L)	0.14	0.02	0.37	—
Epilimnetic chlorophyll (µg/L)	3.77	1.89	5.86	—
Epilimnietic TN (mg/L)	0.20	0.20	0.37	—
Epilimnetic TP (µg/L)	7.89	7.80	11.93	—
TSI_{secchi}	46.2	50.0	48.0	—
$TSI_{chlorophyll}$	43.6	36.9	47.9	—
TSI_{TP}	33.9	33.8	39.9	—
Trophic classification*	MESOTROPHIC	OLIGOTROPHIC	MESOTROPHIC	—
PTOX cyanobacteria found?	—	—	—	YES
Toxin testing recommended?	—	—	—	NO

**according to $TSI_{chlorophyll}$ **Samples for epilimnetic chlorophyll, TN, and TP collected by TWCWC on 15 July 2023. Profile and plankton data collected by PLEON on 17 July 2023.*

Table 3: Summary of Little Twin Lake in 2023

	17 June	**17 July****	**19 Aug**
Thermally stratified?	YES	YES	YES
Epilimnion depth (m)	3	3	4
Metalimnion depth (m)	8	9	9
Secchi depth (m)	3.5	3	3
Vertical extinction coefficient (k)	—	0.97	—
$Z_{10\%}$ (m)	—	2.38	—
$Z_{1\%}$ (m)	—	4.76	—
Mean hypolimnetic DO (mg/L)	0.73	0.06	0.72
Epilimnetic chlorophyll (μg/L)	1.35	2.66	3.24
Epilimnietic TN (mg/L)	0.21	0.22	0.26
Epilimnetic TP (μg/L)	8/18	6.78	7.44
TSI_{secchi}	41.9	44.2	44.2
$TSI_{chlorophyll}$	33.6	40.2	42.2
TSI_{TP}	34.4	31.7	33.1
Trophic classification*	OLIGOTROPHIC	MESOTROPHIC	MESOTROPHIC

**according to $TSI_{chlorophyll}$ ** Samples for epilimnetic chlorophyll, TN, and TP collected by TWCWC on 15 July 2023. Profile and plankton data collected by PLEON on 17 July 2023*

Table 4: Summary of Walker Lake in 2023

	18 June	**17 July**	**20 Aug**
Thermally stratified?	YES	YES	PARTIAL
Epilimnion depth (m)	2	0.5	1
Metalimnion depth (m)	5	6	—
Secchi depth (m)	1.4	2	1
Vertical extinction coefficient (k)	—	1.76	—
$Z_{10\%}$ (m)	—	1.31	—
$Z_{1\%}$ (m)	—	2.61	—
Mean hypolimnetic DO (mg/L)	0.17	0.02	—
Epilimnetic chlorophyll (μg/L)	8.25	15.9	19.0
Epilimnietic TN (mg/L)	0.56	0.28	1.029
Epilimnetic TP (μg/L)	34.41	17.64	35.46
TSI_{secchi}	55.2	50.0	60.0
$TSI_{chlorophyll}$	51.3	57.7	59.5
TSI_{TP}	55.2	45.5	55.6
Trophic classification*	EUTROPHIC	MESOTROPHIC	EUTROPHIC

** according to $TSI_{chlorophyll}$*

II. Chemical Profiles

A. Temperature

Big Twin was thermally stratified during all 2023 samplings (Figure 1). The epilimnion, or the well-mixed surface layer, extended to 4 m, 3 m, and 5 m during the June, July, and August samplings, respectively. The average epilimnetic temperature (± standard deviation) in Big Twin was 18.6 °C (±0.00) during the June sampling, 25.6 °C (±0.48) during the July sampling, and 22.3 °C (±0.08) during the August sampling. The metalimnion, or middle layer of rapid temperature change, extended to 7 m, 8 m, and 8 m during the June, July, and August samplings, respectively.

Little Twin was thermally stratified during all 2023 samplings (Figure 1). The epilimnion extended to 3 m, 3 m, and 4 m during the June, July, and August samplings, respectively. The average epilimnetic temperature (± standard deviation) in Little Twin was 19.4 °C (±0.06) during the June sampling, 26.5 °C (±0.79) during the July sampling, and 23.1 °C (±0.39) during the August sampling. The metalimnion extended to 8 m, 9 m, and 9 m during June, July, and August samplings, respectively.

Walker was thermally stratified during the June samling and partially stratified (i.e., no hypolimnion was delineated) during the July and August sampling (Figure 1). The epilimnion extended to 2 m, 0.5 m, and 1 m during the June, July and August samplings, respectively. The average epilimnetic temperature (± standard deviation) in Walker was 20.4 °C (±0.72) during the June sampling, 25.2 °C (±0.28) during the July sampling, and 22.6 °C (±0.42) during the August sampling. The metalimnion extended to 5.5 m during the June sampling and extended to the deepest sampling points during the July (6.5 m) and August (6 m) samplings.

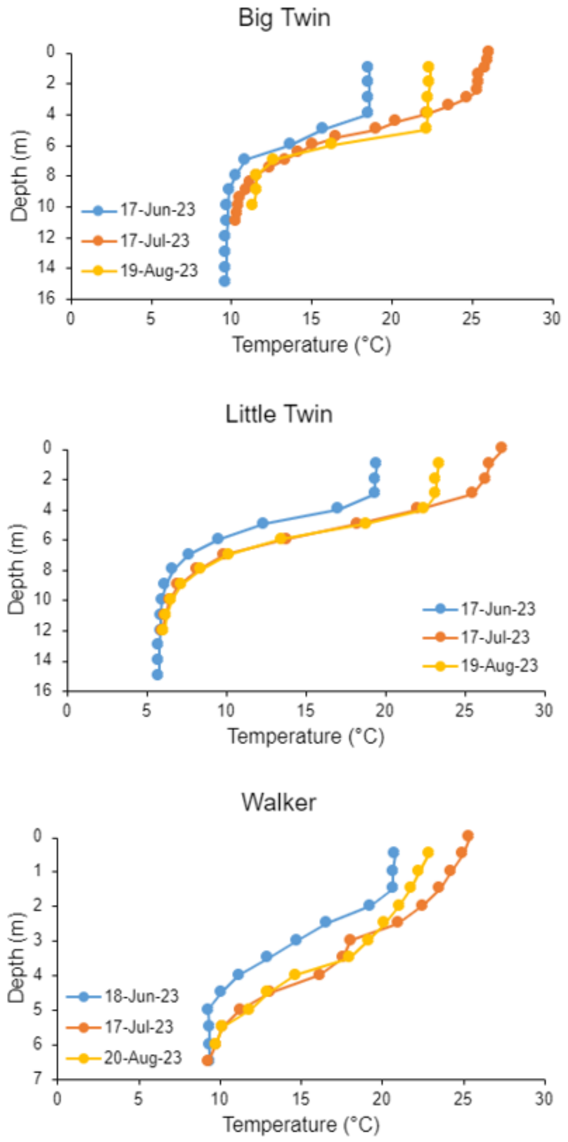


Figure 1: Temperature depth profiles for TWCWC lakes during the summer of 2023. Note scale difference among Y-axes.

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 July and August samplings.

B. Dissolved Oxygen

Big Twin was oxygenated through the epilimnion during all 2023 samplings (Figure 2). Dissolved oxygen concentration (DO) declined through the metalimnion during all samplings. Average DO concentration in the hypolimnion, or deep water, was 0.14 mg/L during the July sampling, and 0.37 mg/L during the August sampling. The depth at which DO concentration was below 2 mg/L, the threshold for oxygen depletion (called hypoxia), was 6 m, 5.5 m, and 7 m during the June, July, and August sampling, respectively.

Little Twin was also oxygenated through the epilimnion during all 2023 samplings (Figure 2) with maximum DO in the metalimnion (peak at 6 m) on all dates. DO concentration declined at depths below these maxima. Average DO in the hypolimnion was 0.73 mg/L during the June sampling, 0.06 mg/L during the July sampling, and 0.72 mg/L during the August sampling. The depth at which hypoxia occurred was 10 m (June), 9 m (July), and 8 m (August).

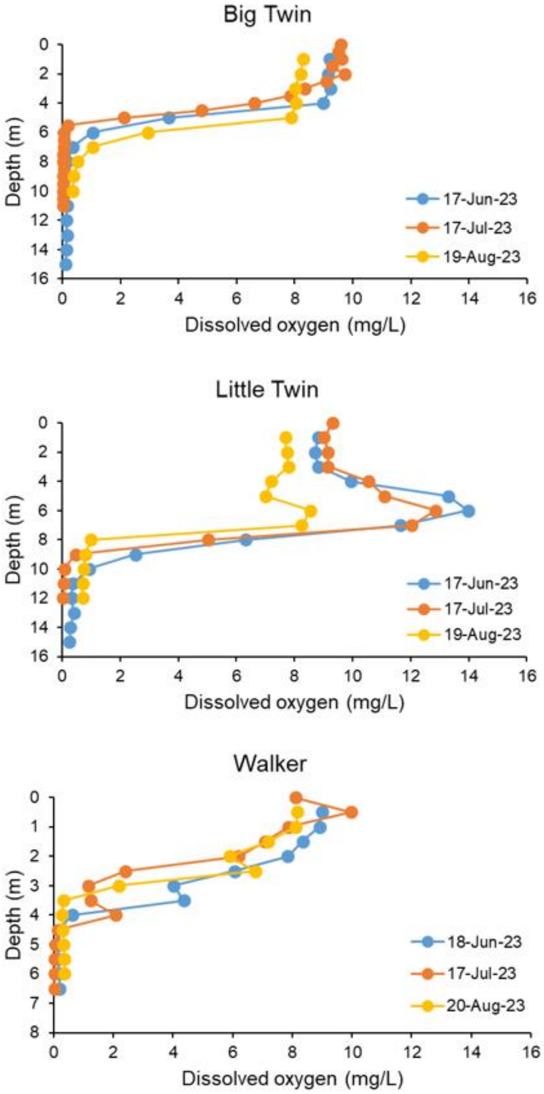


Figure 2: Dissolved oxygen profiles for TWCWC lakes during the summer of 2023. Note scale differences among the Y-axes.

As in the Twin lakes, Walker Lake was oxygenated through the epilimnion and DO generally declined through the metalimnion during all 2023 samplings (Figure 2). Average hypolimnetic DO concentration was 0.17 mg/L during the June sampling. A hypolimnion was not delineated during the July and August samplings, but the DO concentration at the deepest sampling points were 0.02 mg/L and 0.35 mg/L, respectively.

The DO profiles observed in the Twin and Walker lakes are typical. DO is often greater 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 and increased through the deeper waters (Figure 3). Conductivity ranged from 71.4-117.8 µS/cm during the June sampling, from 65.2-81.7 µS/cm during the July sampling, and from 68.2-144.6 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.

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 increased in the deep waters during June and August but was less than the surface waters during the July sampling. Conductivity ranged from 127.3-154.4 µS/cm during the June sampling, from 94.5-135.3 µS/cm during the July sampling, and from 120.2-158.9 µS/cm during the August sampling.

Conductivity of Walker was generally stable in the epilimnion, decreased in the metalimnion (in June and July), and increased in the deep waters (Figure 3). Conductivity ranged from 59.5-106.2 µS/cm during the June sampling, from 47.3-133.4 µS/cm during the July sampling, and from 50-212.4 µS/cm during the August sampling.

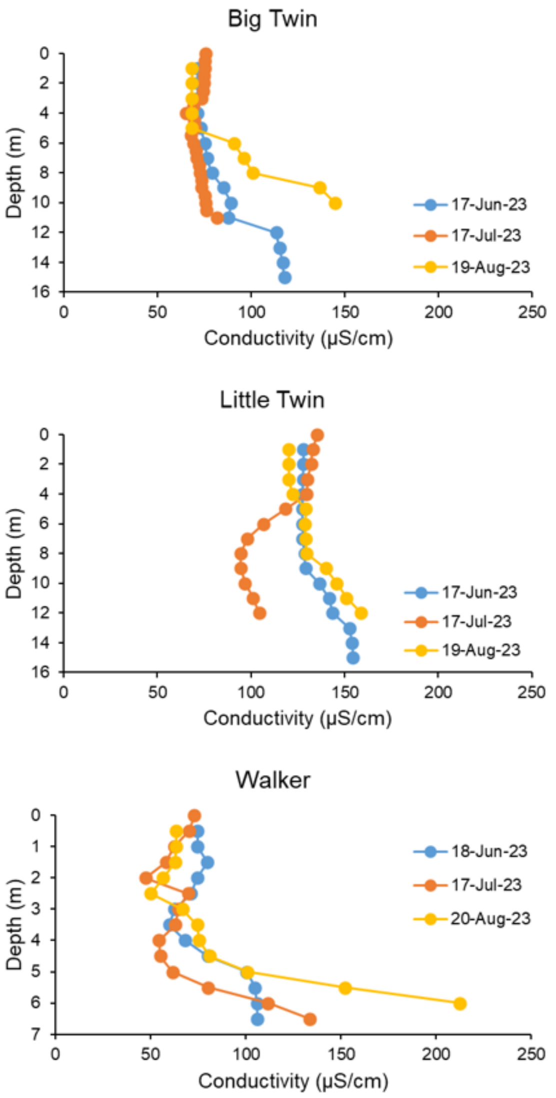


Figure 3: Conductivity depth profiles for TWCWC lakes during the summer of 2023. Note scale differences among the Y-axes.

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 2023 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 6.22-7.48 during the June sampling, from 6.49-7.31 during the July sampling, and from 6.62-8.04 during the August sampling (Figure 4). pH in Big Twin decreased most rapidly in the epilimnion and continued in the shallower depths of the metalimnion. For all samplings, pH increased and stabilized near neutral (7-7.5) through the hypolimnion.

pH in Little Twin ranged from 6.16-7.43 during the June sampling, from 6.43-7.3 during the July sampling, and from 6.29-7.37 during the August sampling (Figure 4). Little Twin pH during the June and August samplings was generally stable through the epilimnion, declined through the metalimnion, and approached neutral (6.5-7) through the metalimnion. The July sampling had a steep drop in pH early in the epilimnion (0 m -1 m), after which pH increased through the profile.

pH in Walker ranged from 6.13-7.48 during the June sampling, from 6.75-8.45 during the July sampling, and from 6.1-7.7 during the August sampling (Figure 4). pH in Walker generally declined through the epilimnion and upper metalimnion and stabilized through the deeper waters.

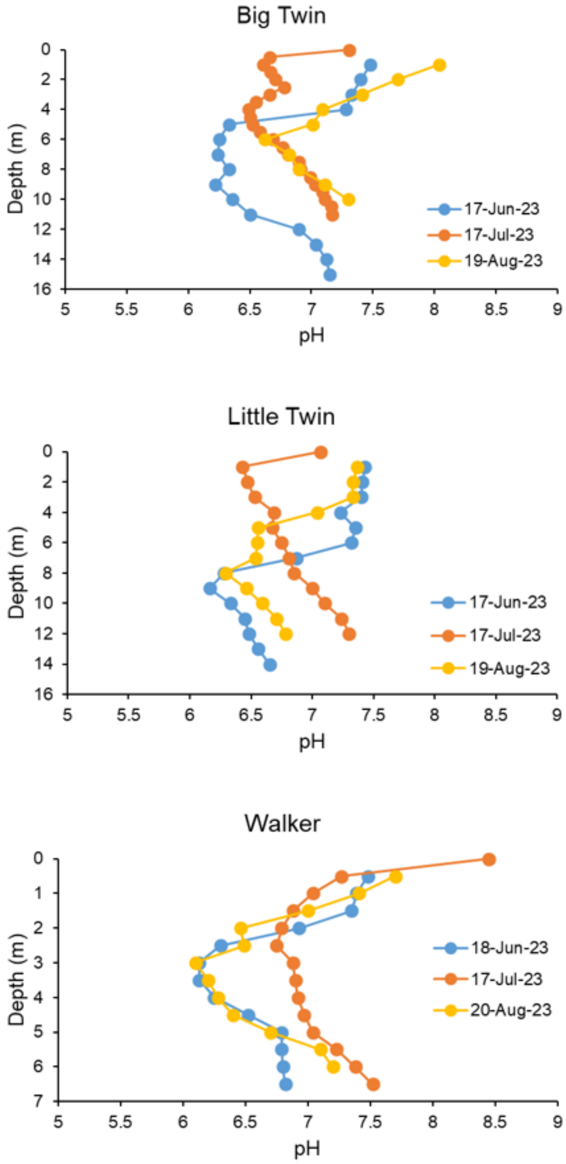


Figure 4: pH depth profiles for TWCWC lakes during the summer of 2023. Note scale differences among Y-axes.

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 within this range. 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

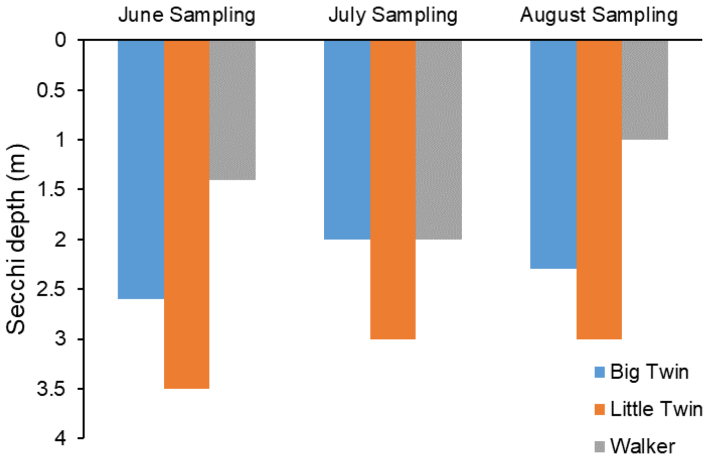


Figure 5: Secchi Depth for TWCWC lakes during summer of 2023. Sampling dates are summarized in Table 1.

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]:

$$TSI_{secchi} = 60 - 14.41 \times \ln\left(Secchi\ depth\right)$$

Secchi depth in Big Twin was 2.6 m, 2 m, and 2.3 m during the June, July, and August samplings, respectively (Figure 5). $\text{TSI}_{\text{Secchi}}$ of Big Twin across these samplings was 46.2, 50.0, and 48.0, respectively, classifying Big Twin as mesotrophic for June and August samplings and eutrophic for the July sampling.

Secchi depth in Little Twin was consistently deeper than other TWCWC lake in 2023, with Secchi depths of 3.5 m, 3 m, and 3 m during the June, July, and August sampling, respectively (Figure 5). $\text{TSI}_{\text{Secchi}}$ of Little Twin across these samplings was 41.9, 44.2, and 44.2, respectively, classifying Little Twin as mesotrophic during all sampling dates.

Walker was generally the least clear TWCWC lake on all 2023 sampling dates (Figure 5). Secchi depth in Walker was 1.4 m, 2 m, and 1.0 m during the June, July, and August sampling, respectively. $\text{TSI}_{\text{Secchi}}$ of Walker across these samplings was 55.2, 50.0, and 60.0, 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 2023 sampling. Little Twin was the clearest of the TWCWC lakes at this time ($k = 0.73$, $Z_{10\%} = 3.18$, $Z_{1\%} = 6.36$), followed by Big Twin ($k = 1.13$, $Z_{10\%} = 2.03$, $Z_{1\%} = 4.06$), and Walker ($k = 1.76$, $Z_{10\%} = 1.31$, $Z_{1\%} = 2.61$).

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 the surface (0.5 m), composite, and hypolimnion sample (if taken; Appendix I).

Chla concentration at 0.5 m in Big Twin ranged from 1.89 µg/L to 5.86 µg/L over the 2023 samplings (Figure 6). The greatest epilimnetic chla concentration occurred in August and the lowest concentration was in July. Composite samples had similar concentrations to epilimnetic samples collected during June and August. The composite sample collected in July has a higher concentration than both the 0.5 m and hypolimnion samples, suggesting that algae were more densely concentrated in the metalimnion on that date. The hypolimnion sample in August had nearly double the chla concentration of the 0.5 m sample, suggesting algae was more concentrated at deeper depths in August. Hypolimnion samples were not collected in June.

Chla concentration at 0.5 m in Little Twin ranged from 1.36 µg/L to 3.25 µg/L over the 2023 samplings (Figure 6). As in Big Twin, algal abundance in the epilimnion was greatest during the August sampling. Chla concentrations nearly double between June and July and composite samples had a greater concentration for all months sampled; additionally, hypolimnion samples from July and August were below detection and are therefore not shown. With an increase in chla concentration from 0.5 m to composite samples each month, it is likely algae was more concentrated in the metalimnion.

Walker was the most productive of the TWCWC lakes during the summer of 2023; note the larger scale for the Y-axis for chla concentration (Figure 6). Chla concentration at 0.5 m in Walker ranged from 8.25 µg/L to 18.98 µg/L with chla concentrations increasing over the summer. The chla concentration at 0.5 m was similar to that of the composite samples in June and August. In July, the concentration at 0.5 m was higher than that of the composite sample, but the hypolimnion sample taken was over 4x more concentrated than the 0.5 m sample. This suggests greater algal biomass at deeper depths and near sediments.

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

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

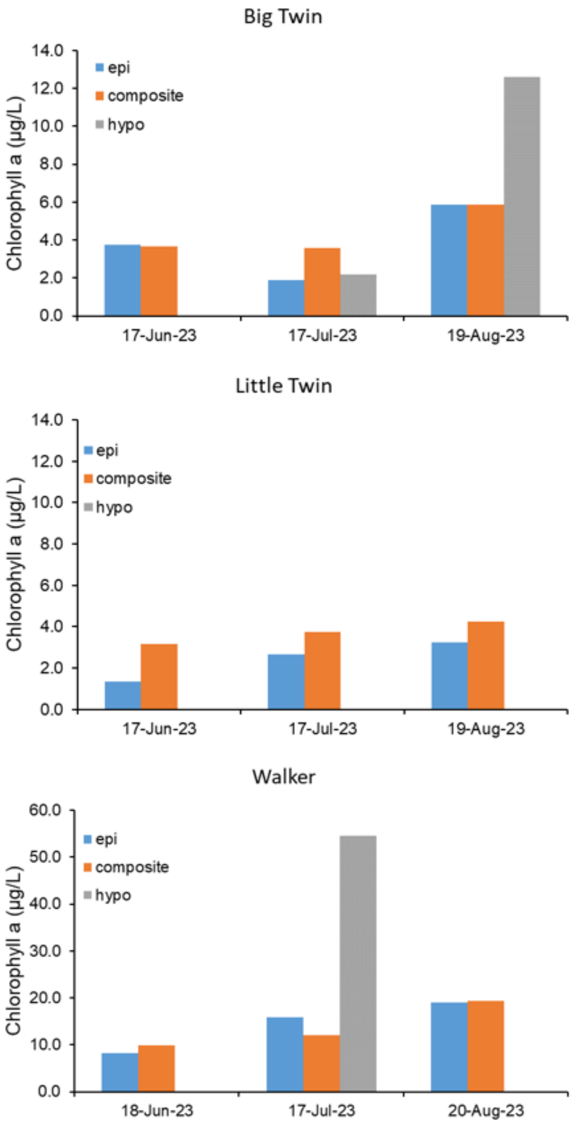


Figure 6: Chlorophyll-a concentrations in TWCWC lakes during 2023. Note scale differences among Y-axes and sampling dates among lakes (summarized in Table 1).

The $TSI_{chlorophyll}$ of Big Twin was 43.6, 36.9, and 47.9 during the June, July, and August sampling, respectively, classifying Big Twin as oligotrophic during July, and mesotrophic during June and August (Table 5).

The $TSI_{chlorophyll}$ of Little Twin was 33.6, 40.2, and 42.2 during the June, July, and August sampling, respectively, classifying Little Twin as oligotrophic during June, oligo-mesotrophic in July and mesotrophic during August (Table 5).

The TSI$_{chlorophyll}$ of Walker was 51.3, 57.7, and 59.5 during the June, July, and August sampling, respectively, classifying Walker as eutrophic through the summer (Table 5).

V. Nutrient Results

A. Total nitrogen

Total nitrogen concentration (TN) in samples collected from 0.5 m in Big Twin ranged from 0.20 mg/L to 0.30 mg/L and generally increased over the course of the summer of 2023 (Figure 7). TN was consistently greater in composite or hypolimnion samples compared to surface samples (by at least 2x in the case of hypolimnetic samples). Hypolimnetic TN also increased from July to August (no hypolimnion sample was collected in June).

TN in samples collected from 0.5 m in Little Twin ranged from 0.21 mg/L to 0.26 mg/L during 2023 with a slight increase from month to month (Figure 7). As in Big Twin, composite and hypolimnion samples had greater TN compared to surface samples in Little Twin (by nearly 2x in the case of the hypolimnetic samples). TN in hypolimnetic samples increased from July to August (no hypolimnion sample was collected in June).

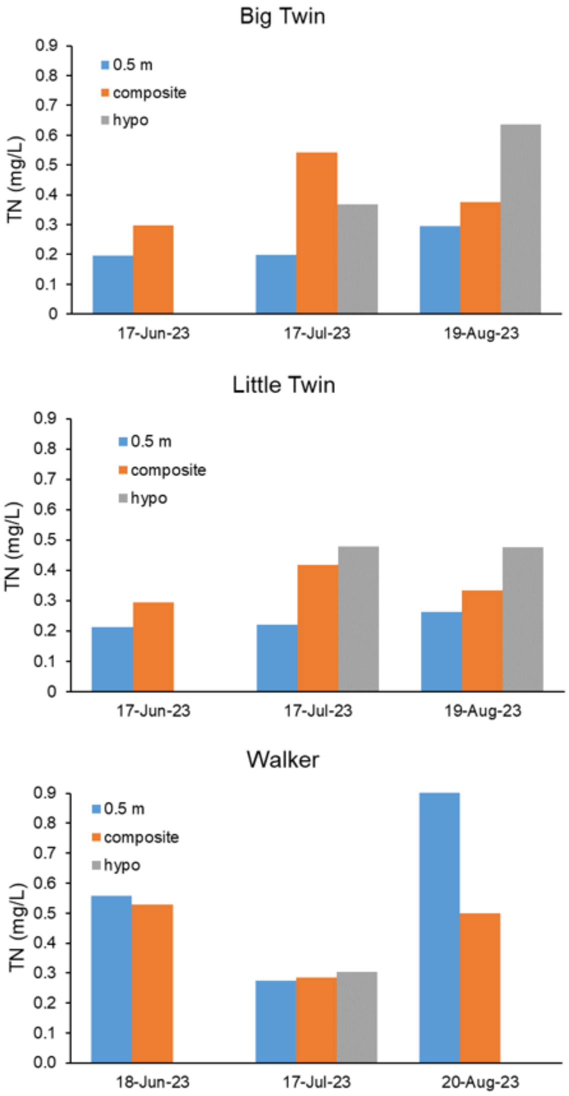


Figure 7: TN concentrations of water samples collected at varying depths for TWCWC lakes for the summer of 2023. Sampling dates summarized in Table 1.

TN in samples collected from 0.5 m in Walker ranged from 0.28 mg/L to 1.029 mg/L during the summer of 2023 (Figure 7). TN during the August sampling was more than 2x that of the June and July samplings. Unlike the Twin lakes, TN in samples containing deeper water was similar to or less than that in surface samples.

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 threshold of 3 mg/L nitrate (one form of nitrogen) used by Penn State Extension to indicate nitrogen pollution[1].

B. Total phosphorus

Total phosphorus concentration (TP) in samples collected from 0.5 m in Big Twin ranged from 7.89 µg/L to 11.93 µg/L during the 2023 samplings (Figure 8). The greatest epilimnetic TP concentration occurred during the August sampling and was approximately 4 µg/L greater than that of the June and July samplings. TP concentrations in the hypolimnion were greater than those of the 0.5 m and composite samples suggesting TP was more concentrated at deeper depths.

TP in samples collected from 0.5 m in Little Twin ranged from 6.77 µg/L to 8.18 µg/L across all 2023 samplings (Figure 8). The August sampling had the highest TP concentrations. Hypolimnetic TP concentrations in July were more than 4x greater than that of the epilimnetic samples. TP in the hypolimnion during the August sampling were also greater than epilimnetic samples, but to a lesser degree.

Walker was generally the most phosphorus-rich TWCWC lake (Figure 8). TP in samples from 0.5 m in Walker ranged from 17.29 µg/L to 35.46 µg/L during the 2023 samplings with similar high epilimnetic TP during the June and August samplings. TP in the composite sample was quite high during the June sampling (41.70 µg/L).

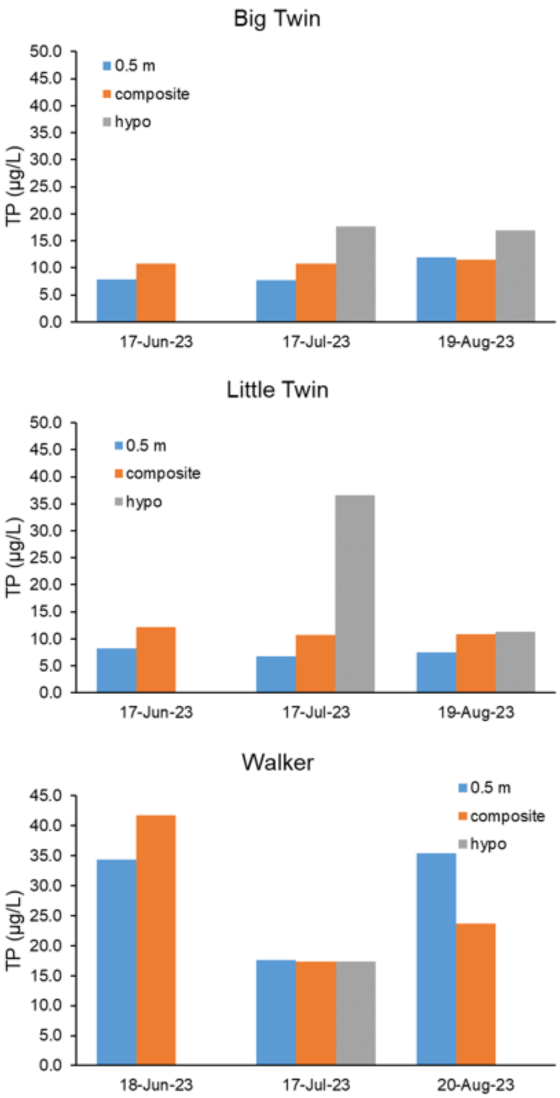


Figure 8: TP concentrations at varying depths for TWCWC lakes during the summer of 2023. Sampling dates are summarized in Table 1.

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 in Big and Little Twin were generally below the 25 µg/L threshold for nutrient pollution suggested by Penn State Extension[1] during 2023 samplings while epilimnetic TP in Walker approached or exceeded this threshold. However, hypolimnetic TP in the Twin lakes was closer to, and sometimes 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 hypolimnetc samples exceeding that of surface samples in TWCWC lakes.

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

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

TSI_{TP} of Big Twin was 33.9, 33.8, and 39.9 during the June, July, and August samplings, respectively. TSI_{TP} classified Big Twin as oligotrophic during all 2023 samplings (Table 5).

TSI_{TP} of Little Twin was 34.4, 31.7, and 33.1 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 55.2, 45.5, and 55.6 during the June, July, and August sampling respectively. TSI_{TP} classified Walker as eutrophic during June and August and mesotrophic in July (Table 5).

C. Dissolved organic carbon

Dissolved organic carbon concentration (DOC) in Big Twin was 4.93 mg/L, 3.94 mg/L, and 4.83 mg/L in samples collected from 0.5 m during the 17 June, 15 July, and 19 August samplings, respectively. DOC in composite samples was consistently greater than surface samples in this lake with 4.60 mg/L, 6.14 mg/L, and 4.93 mg/L during the June, July, and August samplings, respectively. DOC of the July composite sample was quite high and may have been contaminated.

DOC in Little Twin was 3.84 mg/L, 3.98 mg/L, and 3.15 mg/L in samples collected from 0.5 m during the 17 June, 15 July, and 19 August samplings, respectively. Unlike Big Twin, Little Twin composite samples had less or similar DOC compared to the surface samples with 3.75 mg/L, 3.53 mg/L, and 3.16 mg/L during the June, July, and August samplings, respectively.

DOC in Walker was 4.23 mg/L, 5.06 mg/L, and 6.04 mg/L in samples collected from 0.5 m during the 18 June, 17 July, and 20 August samplings, respectively. DOC in composite samples was 4.24 mg/L, 5.40 mg/L, and 5.75 mg/L during the June, July, and August samplings, respectively.

The pool of DOC in lakes includes soluble organic compounds that wash in from the watershed, biproducts of the decomposition of aquatic plants and animals, and molecules that are synthesized within the water column[4]. DOC concentration is affected by the frequency and intensity of precipitation as well as the chemistry and structure of watershed soils.

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 17 July 2023. Walker was not sampled for zooplankton.

Zooplankton numbers in both lakes was dominated by rotifers, which made up 88% and 93% 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 a lesser percentage of total zooplankton biomass (28% biomass in Big Twin, 9% biomass in Little Twin).

Copepods made up 2% and 10% of zooplankton density in Big Twin and Little Twin, respectively. Other larger organisms such as Cariboridae (listed as other zooplankton in

Table 6: Zooplankton community in the Twin Lakes on 17 July 2023 (averages of 2 samples).

	Big Twin				**Little Twin**			
	Density (cells/L)	**Relative density (%)**	**Biomass (µg/L)**	**Relative biomass (%)**	**Density (cells/L)**	**Relative density (%)**	**Biomass (µg/L)**	**Relative biomass (%)**
PROTOZOA	**11**	**9%**	**0**	**0%**	**0**	**0%**	**0**	**0%**
Ciliophora	11		0		0		0	
Mastigophora	0		0		0		0	
Sarcodina	0		0		0		0	
ROTIFERA	**117**	**88%**	**15**	**28%**	**46**	**93%**	**4**	**7%**
Ascomorpha	0		**0**		0		0	
Asplanchna	1		**5**		0		0	
Conochilus	18		**1**		3		0	
Filinia	0		**0**		0		0	
Hexarthra	1		**0**		0		0	
Kellicottia	1		**0**		2		0	
Keratella	37		**3**		25		2	
Lepadella	1		**0**		0		0	
Polyarthra	55		**6**		15		1	
Trichocerca	4		**1**		1		0	
COPEPODA	**3**	**2%**	**8**	**14%**	**5**	**10%**	**13**	**26%**
Copepoda-Cyclopoida	2		4		2		6	
Cyclops	1		3		1		3	
Mesocyclops	1		1		1		3	
Copepoda-Calanoida	0		0		0		0	
Other Copepoda-Naupli	2		4		3		8	
CLADOCERA	**1**	**1%**	**1**	**2%**	**2**	**4%**	**4**	**8%**
Bosmina	1		1		1		1	
Ceriodaphnia	0		0		1		2	
Daphnia catawba	0		0		0		1	
OTHER ZOOPLANKTON	**0**	**0%**	**30**	**56%**	0	**0%**	**30**	**59%**
TOTAL	**132**		**54**		**49**		**51**	

Table 6) and Cladocerans were found in relatively low densities but accounted for 72% and 93% of biomass in Big Twin and Little Twin, respectively. These taxa are essential food sources for fish and are important algae grazers (particularly cladocerans).

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 12.5 and 11.5, respectively and average diversity (Shannon-Wiener Index) was 0.67 and 0.66, respectively.

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 17 July 2023.

Chlorophyta were the numerically dominant group in Big Twin Lake (55% phytoplankton density) while Cyanophyta (cyanobacteria) were the dominant group Little Twin Lake (85% phytoplankton density). Bacillariophyta, Cryptophyta, and Cyanophyta density made up at least 10% of phytoplankton density in Big Twin. In Little Twin, Chrysophyta was the second most dominant group making up 13% of phytoplankton density. Other groups of algae were in low abundance (Table 7).

The Cyanophyta, or cyanobacteria, 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 *Limnothrix*, and *Planktolygbya*. All of these genera are capable of producing toxins that can be harmful to humans and pets.

Average phytoplankton taxonomic richness in Big Twin was 11.5 and average diversity (measured using the Shannon-Wiener Index) was 0.93. Planktonic richness in Little Twin was 9.5 and diversity was 0.32.

Table 7: Phytoplankton community in the Twin Lakes on 17 July 2023 (averages of 2 samples).

	Big Twin				**Little Twin**			
	Density (cells/ml)	**Relative density (%)**	**Biomass (µg/ml)**	**Relative biomass (%)**	**Density (cells/ml)**	**Relative density (%)**	**Biomass (µg/ml)**	**Relative biomass (%)**
BACILLARIOPHYTA	**107**	**10%**	**42**	**4%**	**174**	**0.8%**	**114**	**1%**
Centric Diatoms	**88**		**26**		**0**		**0**	
Araphid Pennate Diatoms	**19**		**15**		**174**		**114**	
CHLOROPHYTA	**601**	**55%**	**249**	**22%**	**161**	**0.8%**	**456**	**5%**
Coccoid/Colonial Chlorophytes	**375**		**156**		**42**		**4**	
Filamentous Chlorophytes	**214**		**43**		**0**		**0**	
Desmids	**13**		**50**		**119**		**452**	
CHRYSOPHYTA	**64**	**6%**	**191**	**17%**	**2667**	**13%**	**8002**	**90%**
Flagellated Classic Chrysophytes	**64**		**191**		**2667**		**8002**	
CRYPTOPHYTA	**167**	**15%**	**608**	**53%**	**76**	**0.4%**	**64**	**0.7%**
CYANOPHYTA	**126**	**11%**	**25**	**2%**	**17964**	**85%**	**180**	**2%**
Filamentous Nitrogen Fixers	**126**		**25**		**0**		**0**	
Filamentous Non-Nitrogen Fixers	**0**		**0**		**17964**		**180**	
EUGLENOPHYTA	**38**	**3%**	**38**	**3%**	**28**	**0.1%**	**28**	**0.3%**
TOTAL	**1102**		**1153**		**21070**		**8843**	

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.

TWCWC collected multiple samples from Big Twin Lake on 21 August 2023. Samples were collected from wrist depth and were shipped to Greenwater Laboratories for microscopic analysis (Appendix I). Samples were collected from the following locations (notations by TWCWC): Yerdon, Inlet, Rentals/Hoff, TLWoods, Sagamore, and Midway.

PTOX cyanobacteria were not observed in the Yerdon, Inlet, or Rentals/Hoff samples. *Dolichospermum*, a potentially toxigenic genus of cyanobacteria, was observed in low abundances (1-4 filaments per ml) in the TLWoods, Sagamore, and Midway samples. *Dolichospermum* can produce microcystin, cylidrospermopsin, saxitoxin, and anatoxin-a. However, Greenwater Laboratories did not recommend cyanotoxin testing for any of the samples due to the low abundance of PTOX cyanobacteria.

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 2008-2018 were provided by the TWCWC in the form of yearly "state of the lake" reports by FX Browne and/or physical data sheets.

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 2014-2023.

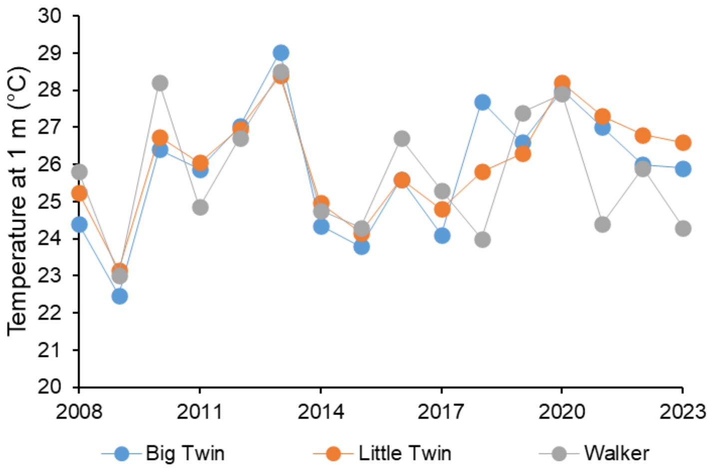


Figure 9: July temperature at 1 m in TWCWC lakes from 2008 to 2023.

TWCWC lakes were generally stratified in the summer months (June, July, August) from 2014-

2023. Surface temperature in TWCWC lakes, while variable, have generally increased over this period, though not with statistical significance (Figure 9).

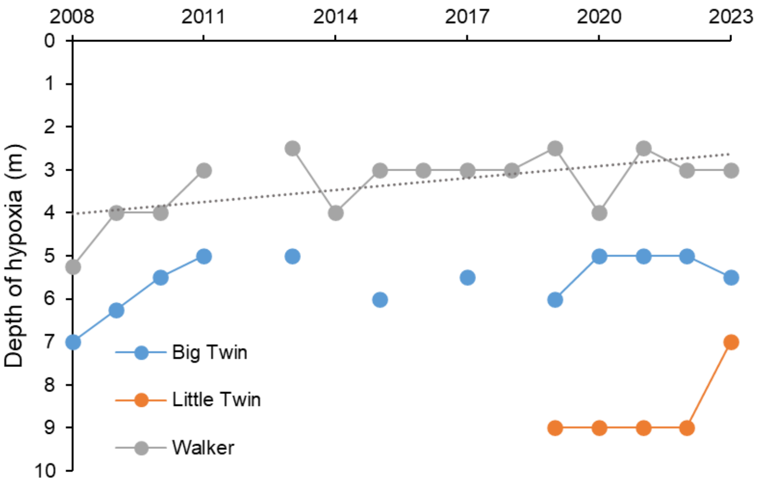


Figure 10: Depth of hypoxia in July for TWCWC lakes from 2008 to 2023.

The TWCWC lakes were generally deplete of oxygen in the hypolimnion during the summer months. 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 10). The trendline for Walker data indicates a significant decrease in the depth of DO depletion. Note that in Figure 10, missing data in Walker Lake indicate that there was no hypoxia while missing data for the Twin Lakes indicate incomplete profiles, not the absence of hypoxia. 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 148.06 µS/cm compared to 85.2 µS/cm and 86.5 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 6.71 compared to 7.03 and 7.24 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

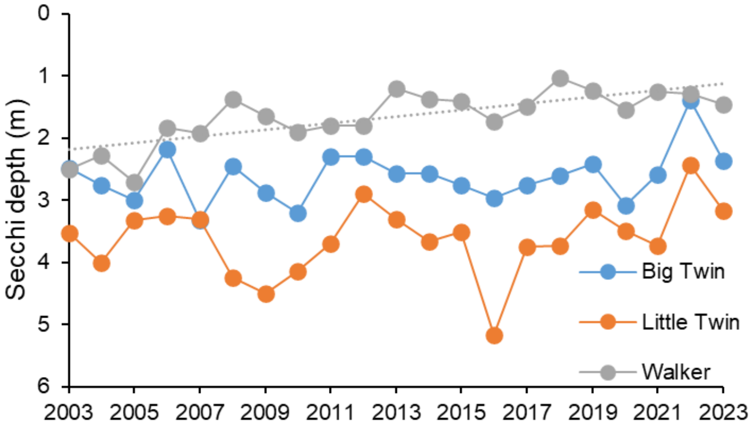


Figure 11: Average summer (June, July, August) Secchi depth for TWCWC lakes from 2003 to 2023.

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

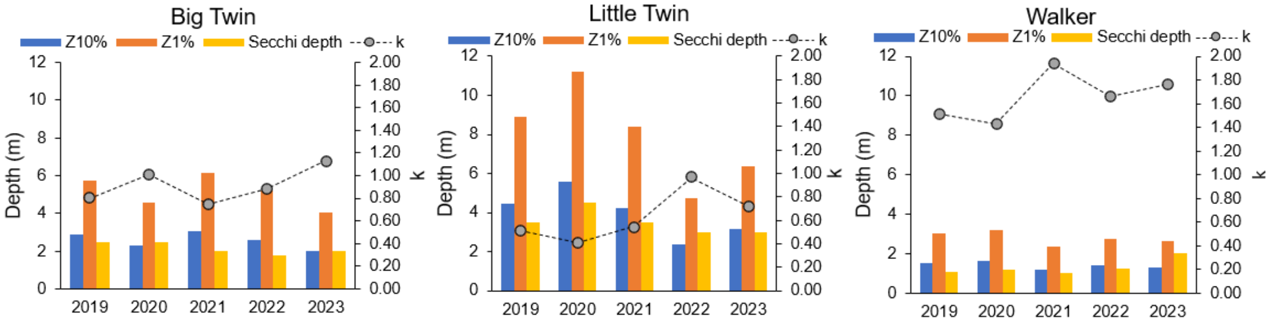


Figure 12: Light attenuation in TWCWC lakes from 2019-2023. Measurements made in July.

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 2003-2023; Figure 11). Secchi depth in Walker decreased over the 9-year dataset (linear regression, $p < 0.01$, $r^2 = 0.58$). In 2022, both Big Twin and Little Twin lakes had their shallowest Secchi depths recorded.

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 0.8-1.2) and Walker (*k* ranged from 1.4-2.0; Figure 12). Transparency in Little Twin decreased from 2020 to 2023.

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 2.4 µg/L to 14.38 µg/L in Walker (Figure 13). Chla concentrations were on the low end of those ranges from 2019 to 2021 in the Twin lakes. However, average summer epilimnetic chla

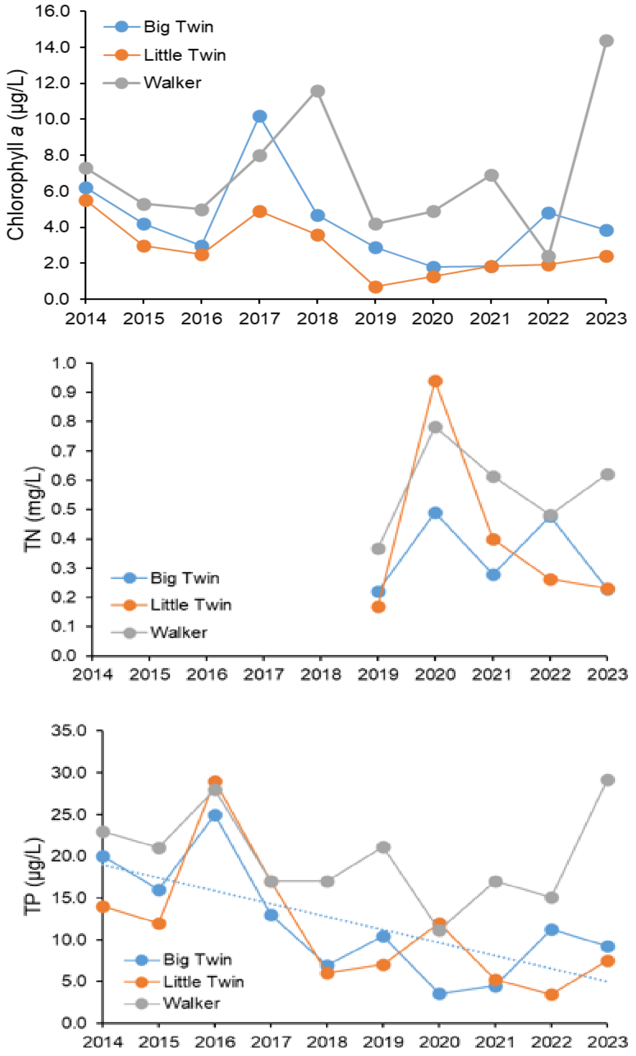


Figure 13: Average epilimnetic chla, TN, and TP in TWCWC lakes from 2014-2023. Dashed line shows linear decline in TP concentration over time in Big Twin.

concentration has been increasing since 2019 in Little Twin. Big Twin saw a relatively dramatic increase in chla concentration in 2022, but has slightly decreased in 2023. Although chla in Walker dramatically decreased in 2022 to the lowest recorded chla concentrations, the highest spike has been recorded for chla in 2023.

E. Nutrients over time

TN has been measured in TWCWC lakes since 2019. Average summer (June, July, August) TN measured in samples collected from 0.5 m ranged from 0.20 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 0.28 mg/L to 1.03 mg/L in Walker (Figure 13). The greatest TN 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 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 35.5 µg/L in Walker (Figure 13). Summer TP generally declined over the 8-year period in all lakes. This decline is statistically significant in Big Twin (linear regression, $r^2 = 0.49$, $p = 0.02$) but not in Little Twin or Walker. In 2023, both Little Twin and Walker have seen an increase in TP, with the latter being more drastic.

F. Trophic status over time

Big Twin and Walker were generally mesotrophic since 2014 and Little Twin was generally oligo-mesotrophic (Figure 14). TSI_{Secchi} was typically greater than

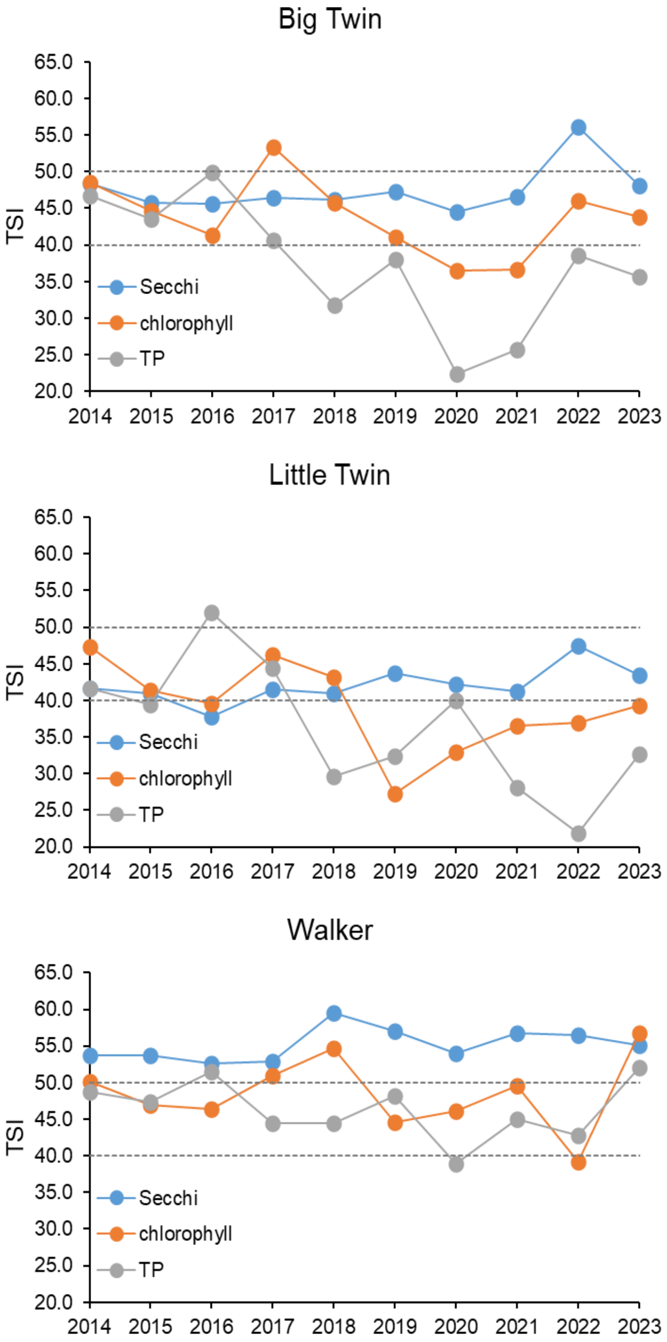


Figure 14: Average summer TSI in TWCWC lakes from 2014-2023 according to Secchi depth, chla, and TP. Horizontal lines show thresholds for oligotrophy (<40), mesotrophy (40-50), and eutrophy (>50).

TSI$_{chlorophyll}$ and TSI$_{TP}$ in all three lakes, particularly since 2018 when TSI$_{chlorophyll}$ and TSI$_{TP}$ began declining. However, these metrics increased in all lakes in recent years.

G. Zooplankton Over Time

PLEON has characterized plankton communities in Big Twin and Little Twin since 2019.

Zooplankton density ranged from 116 to 540 individuals/L in Big Twin and from 52 to 341 individuals/L in Little Twin across the 4-year dataset (Figure 15). Zooplankton were generally less abundant in Little Twin than Big Twin. Zooplankton communities in both lakes were dominated by rotifers across all years.

Average zooplankton richness ranged from 10.5-12.5 in Big Twin and from 9-16.5 in Little Twin over the 4-year dataset (Figure 15). Zooplankton diversity ranged from 0.67-

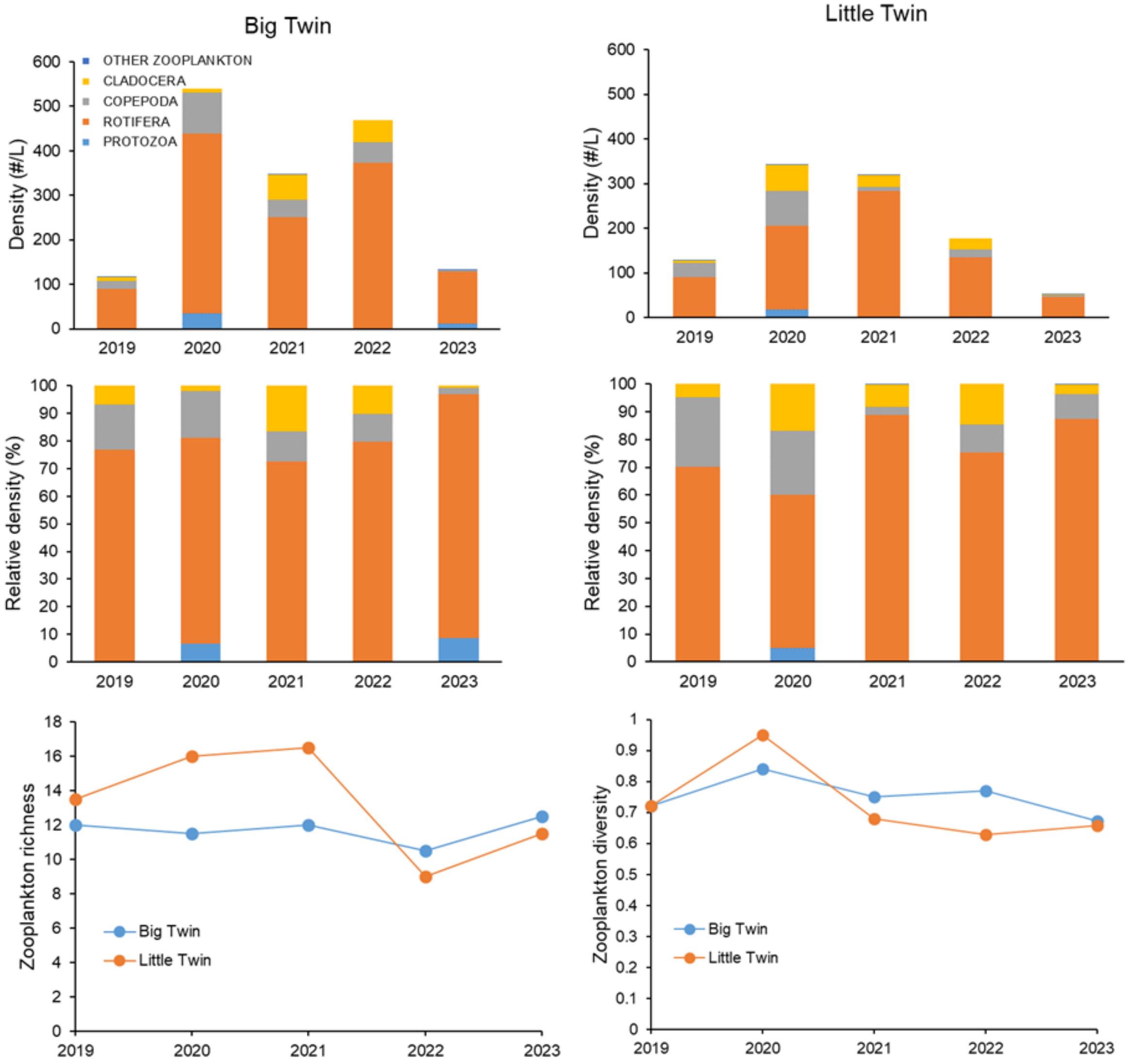


Figure 15: Zooplankton communities in the Twin Lakes from 2019-2023. Samples were collected in July of each year. Bars and symbols are averages of two replicates.

0.84 in Big Twin and from 0.63-0.95 in Little Twin. Average zooplankton length ranged from 0.12 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 16). 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 16). Diversity in Big Twin Lake increased from 2022 to 2023 with Chlorophyta as the most dominant group. More years of sampling are needed to determine if this shift is due to variation or will become more “normal” for Big Twin Lake.

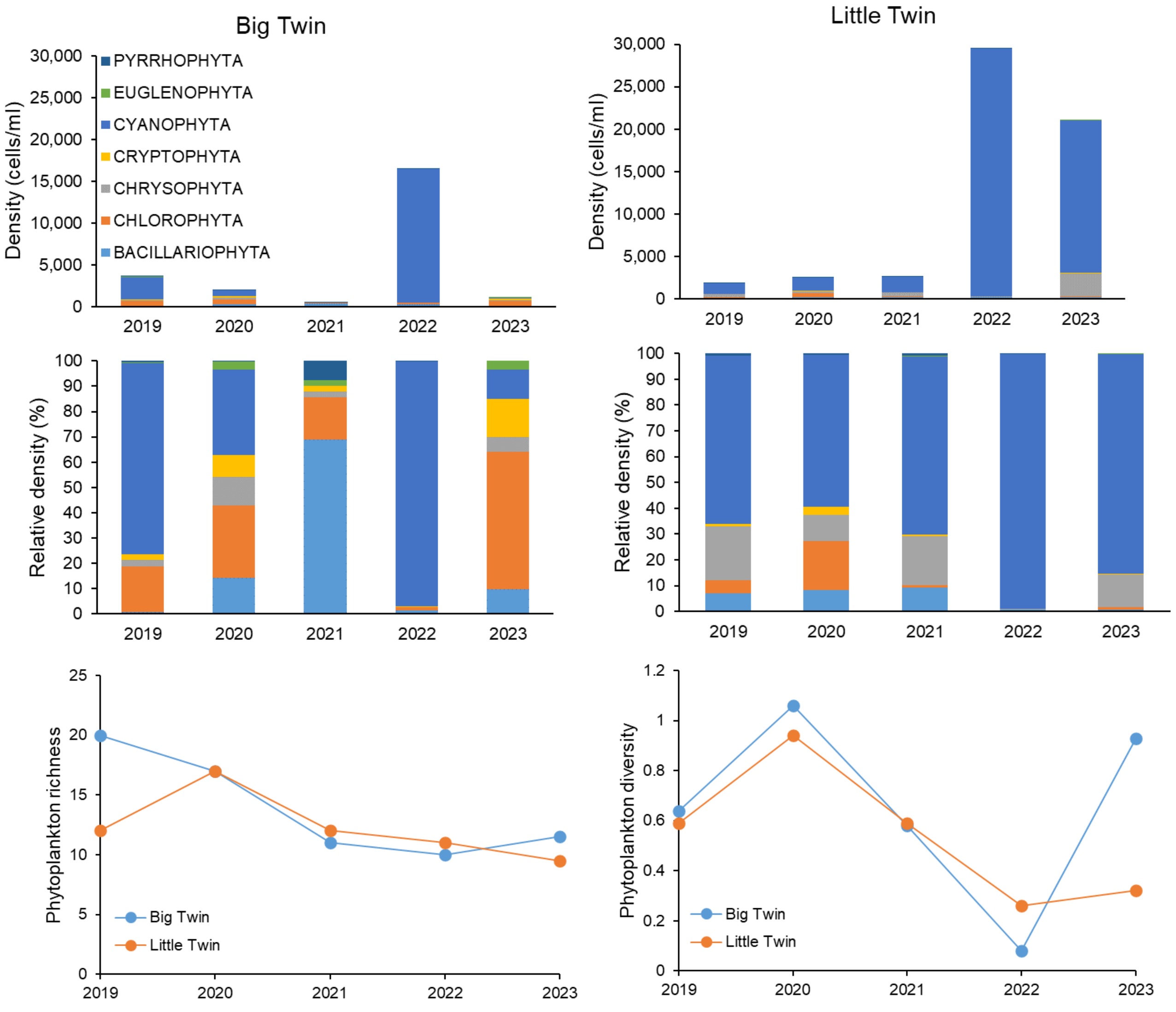


Figure 16: Phytoplankton communities in the Twin Lakes from 2019-2023. Samples were collected in July during each year. Bars and symbols are averages of two replicates.

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. Samples from Big Twin Lake were screened through PLEON in 2021, 2022 and 2023. 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.

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).

The results described above are based on PTOX screening. Data from the comprehensive phytoplankton community analysis may provide more insight. These data are pending for Big Twin and Little Twin.

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*	—
	21 Aug 2023	*PLEON*	*Yerdon*	*Samples collected by TWCWC*	*None found*	—
		PLEON	*Inlet*		*None found*	—
		PLEON	*Rentals/Hoff*		*None found*	—
		PLEON	*TLWoods*		*Dolichospermum*	Testing not recommended
		PLEON	*Sagamore*		*Dolichospermum*	Testing not recommended
		PLEON	*Midway*		*Dolichospermum*	Testing not recommended
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*

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

A. Description of PLEON Lakes

The PLEON dataset consists of 29 lakes in Pike, Wayne, and Monroe Counties. Lakes range in surface area, shoreline, and depth (Figure 17). Big Twin and Little Twin are deeper than the 8.1 m average of the 29 PLEON lakes and Walker is more shallow.

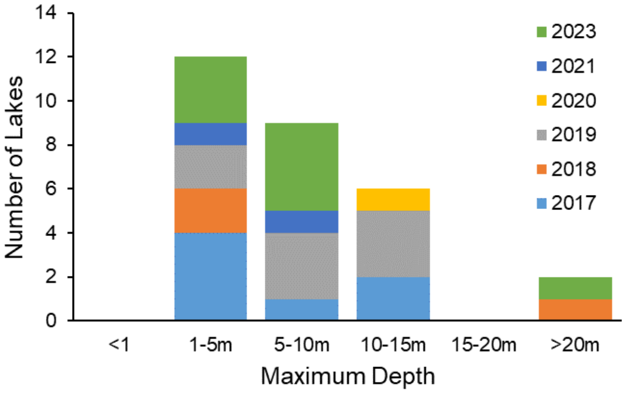


Figure 17: Maximum depth of PLEON lakes. Years refer to the first PLEON sampling year. Not all lakes are sampled every year.

B. Water transparency

PLEON recorded Secchi depth at least once during the summer months (June, July, August) of 2023 in 17 of the 29 lakes. The average summer Secchi depth in these lakes ranged from 0.8 m to 4.75 m with an average of 2.04 m (Figure 18). The average summer Secchi depth of Big Twin and Little Twin were slightly deeper than the PLEON average while average summer Secchi depth in Walker was more shallow than the

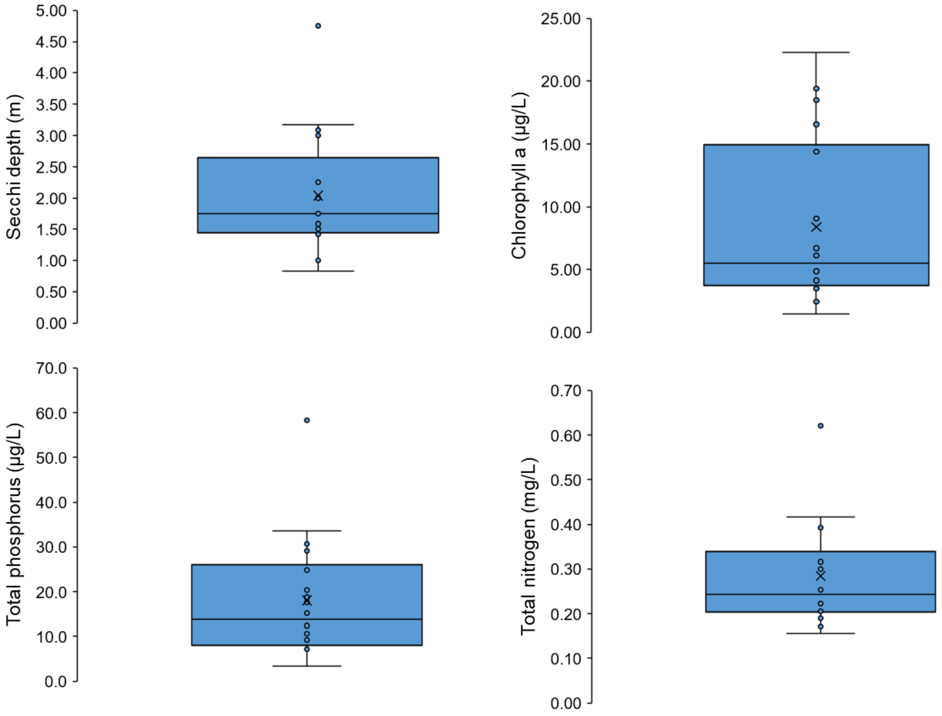


Figure 18: Average summer (J, J, A) Secchi depth, chla, TN, and TP of 18 PLEON lakes monitored in 2023. Lines are medians and X symbols are means. Upper and lower boundaries are the 75th and 25th percentile, respectively. Whiskers show maximum and minimum values. Circles are single measurements or an average if the lake was sampled more than once during the summer. Nutrient concentrations were quantified from 0.5 m depth.

PLEON average.

C. Lake productivity

Lake productivity, as measured by chla concentration at 0.5 m depth, was assessed in 18 PLEON lakes during the summer months (June, July, August) in 2023. Average summer Chla concentration in these lakes ranged from 1.44 µg/L to 22.3 µg/L with an average of 8.38 µg/L (Figure 18). Average summer chla concentration in Big Twin and Little Twin was below the PLEON average while average summer chla concentration in Walker was above the PLEON average.

D. Nutrient concentration

Average summer TN and TP was quantified at 0.5 m depth in 18 PLEON lakes during 2023. TN ranged from 0.16 mg/L to 0.62 mg/L in these lakes, with an average of 0.28 mg/L (Figure 18). Average summer TN in Big Twin and Little Twin were less than the PLEON average while the average summer TN in Walker was greater than the PLEON average.

TP in the 18 PLEON lakes monitored ranged from 3.31 µg/L to 58.34 µg/L in 2023, with an average of 18.1 µg/L (Figure 18). Average summer TP concentration in both Twin lakes was less than the PLEON average while average summer TP in Walker was greater than the PLEON average.

E. Cyanobacteria

Since 2017, PLEON has collected 254 samples for PTOX screening as a part of its formal monitoring program. These samples were collected from 21 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.

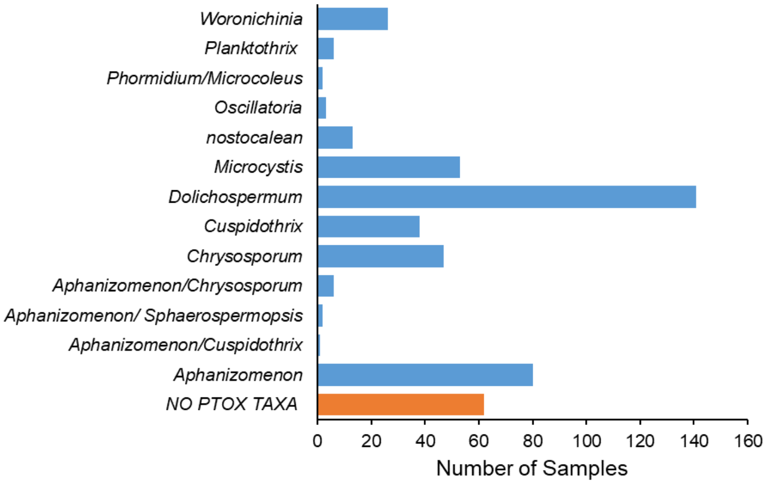


Figure 19: Potentially toxic (PTOX) cyanobacteria genera found in samples collected from PLEON lakes since 2017. PTOX screens were conducted by Greenwater Laboratories.

Ten (possibly 11, some specimens are difficult to identify) PTOX cyanobacteria genera have been found in PLEON samples to date (Figure 19). The most commonly found genera are *Dolichospermum*, followed by *Aphanizomenon* (or *Aphanizomenon*-like). *Chrysosporum*, *Woronichinia*, and *Microcystis* were also common. 62 of the samples (or 24%) did not have PTOX taxa present. Two lakes within the dataset have been consistently free of PTOX taxa, but note 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 31% of the samples and quantifying cylindrospermopsin, anatoxin-a, and/or saxitoxin concentration in 22-23% of the samples. Cyanotoxin quantification is an opt-in service; to date, between 65% and 78% 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)	# above OH threshold**
microcystins/nodularins	78	61	20	9.44	0.16-129	2
cylindrospermopsin	57	37	1	0.07	-	0
anatoxin-a	57	40	0	-	-	0
saxitoxin	58	41	4	0.32	0.15-0.45	0
homoanatoxin-a	1	1	0	-	-	-

**MDL = minimum detection limit*

X. What it all Means: Summary of Key Findings

Several findings from the Twin and Walker lakes 2023 monitoring program should be highlighted:

1. Algae increased over the summer.

Chlorophyll a concentration, a proxy for the amount of algae, increased over the summer in all three lakes. There was a corresponding decrease in water clarity over the same time. A similar trend was observed in 2022, but the 2023 increase was less dramatic, particularly in the Twin Lakes.

In 2022, an increase in phosphorus availability occurred coincident with the increase in algae abundance. This was very clear in Big Twin Lake. There was a similar increase in TP concentration during the late summer in 2023. This increased nutrient availability may have contributed to the increase in algae. This dynamic was most clear in Walker during 2023; there was a clear increase in algae abundance from July to August, with a corresponding increase in TP over the same time.

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. 2023 is the first year that hypolimnion samples were collected. The generally greater TP in hypolimnetic samples compared to surface samples suggests that nutrient regeneration is occurring in Big and Little Twin. However, the overall contribution of internal phosphorus loading relative to other potential sources of phosphorus in these lakes is unknown.

TP depth patterns in Walker were different that the Twin Lakes. In Walker, the TP in samples containing deep water (composite samples) were either similar to or less than that of surface samples in July and August. This suggests either that nutrient regeneration is minimal (unlikely given the consistent hypoxia in this lake), there is a surface source of phosphorus such as runoff or effluent, or that Walker Lake mixes more frequently during the summer than the deeper Twin lakes.

2. Weak correlations between algal abundance and water clarity and between algal abundance and phosphorus availability over the long-term dataset.

The coincident changes in water clarity, algal abundance, and phosphorus availability observed over the past several years in the Twin lakes (as 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, these proposed connections weaken (Figure 20). Average summer Secchi depth was not significantly correlated with average summer chlorophyll a concentration in any of the TWCWC lakes (correlation coefficient $r \leq 0.18$, suggesting <18% of the variation in one variable responds to the variance in the second variable).

Chlorophyll a concentration was more strongly correlated with TP concentration in the Twin Lakes (r = 0.25 in Big Twin Lake, r = 0.36 in Little Twin Lake), suggesting that the amount of algae is responding to the amount of phosphorus in these lakes to some degree. However, these correlations were not statistically significantly in either lake. Chlorophyll and TP were not correlated in Walker lake (r = 0.09).

There are several explanations for weaker correlations between these variables than expected. First, the lack of statistical significance of the correlations between chlorophyll and TP in the Twin Lakes may be due to a small sample size. Ecologically, 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.

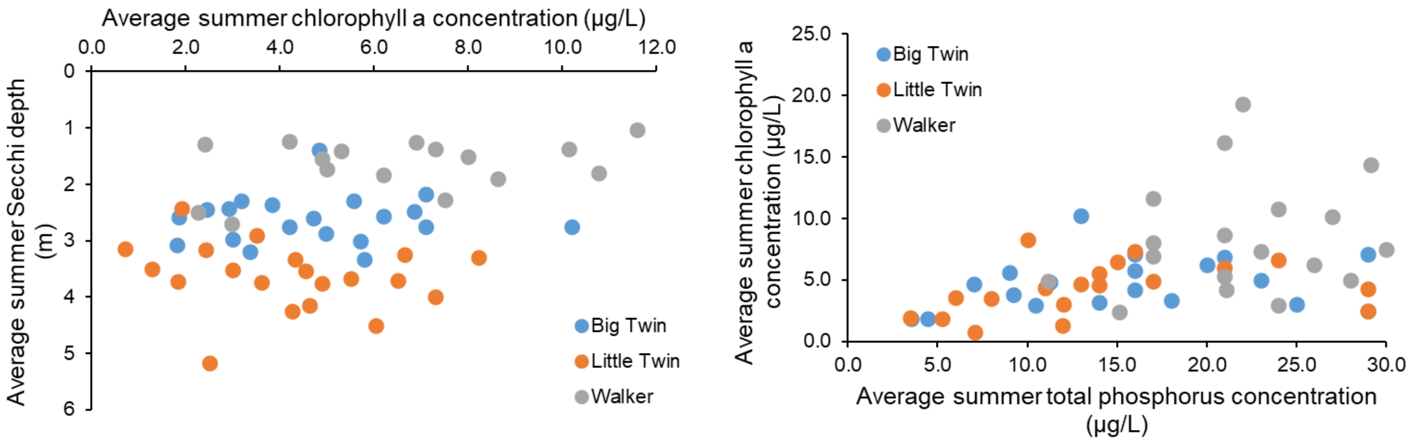


Figure 20: Correlations between average summer Secchi depth and average summer chlorophyll a and between average summer chlorophyll a and average summer TP in TWCWC lakes over the long-term dataset.

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

Water clarity in Walker (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.

There are several lines of evidence supporting this possibility:

1. Potentially toxigenic (PTOX) cyanobacteria has been detected in TWCWC lakes as evidenced by infrequent and inconsistent sampling efforts over the past several years. These screens have shown 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, ***<u>no visible bloom was present in Little Twin or Walker when these samples were taken</u>***. PTOX cyanobacteria counts from a sample collected from Big Twin in June of 2021 also prompted Greenwater Laboratories to recommend cyanotoxin testing.

2. 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.
3. Algal blooms have occurred in Big Twin Lake, most notably in 2022. It is possible, although not known, that this bloom was composed of cyanobacteria.

Based on these factors, TWCWC may want to adopt a more regular and comprehensive HABs monitoring plan. In addition, it would be prudent to consider how to disseminate information regarding HABs exposure risk and HAB testing data to the community.

Algae results of any PLEON sampling 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.

5. Atypical conductivity and pH profiles in Little Twin during July of 2023.

The reason for the “wonkiness” of these profiles is unclear. Surface conductivity and pH may have been affected by the poor air quality experienced in the region during this time. It is possible that residue from the Canadian wildfires altered surface pH. Little Twin may have been more strongly impacted given its size. However, this is only one possibility. Continued monitoring will determine if these atypical profiles become more typical in the future.

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 US EPA method 445.0. Water samples were collected from specific depths 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 12 ml of a 90% acetone solution. Filters were masticated in a tissue grinder before extraction. The extraction took place over 18 hours at 4°C in the dark. Chlorophyll concentration of the extractant was determined via fluorometry (Turner Designs 10AU fluorometer) and corrected for phaeophyton via acidification.

C. Nutrients

Water samples were collected as described above. Water samples were collected in acid washed bottles and kept cold until return to the lab. A 30-40 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)

Water samples were collected and transported as described for nutrient analysis. 40-ml subsamples 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.

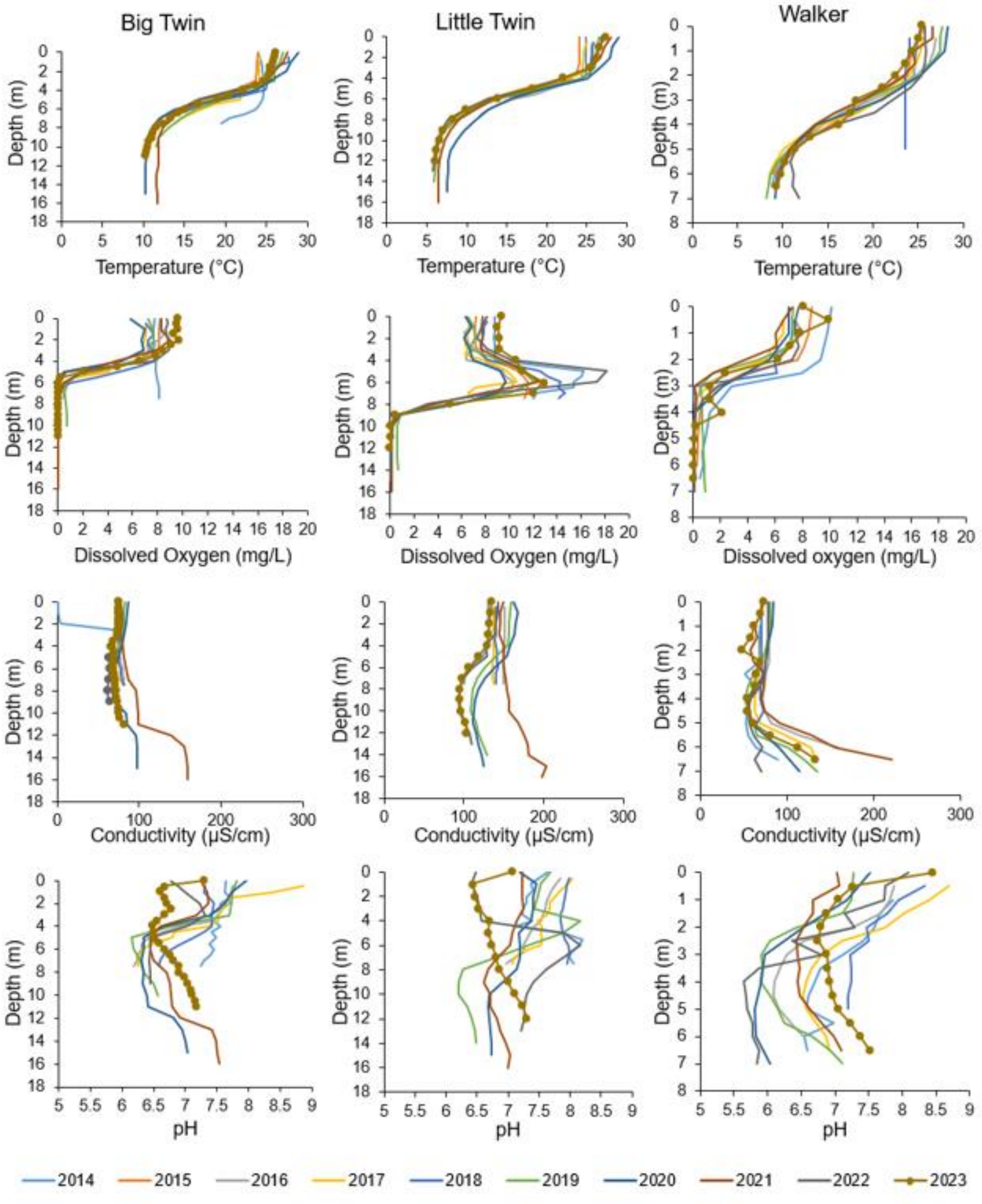
F. Zooplankton and phytoplankton community analysis

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 2014-2023 in TWCWC lakes. Note differences in Y axes among lakes. Brown lines with markers indicate most recent year: 2023.

Appendix III: Literature Cited

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3. Carlson, R. E. 1977. A trophic state index for lakes. Limnology and Oceanography 22(2): 361-369.

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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 PTOX Cyanobacteria Screen 230821 (Big Twin)