To the Twin and Walker Creeks Watershed Conservancy

Report of 2024 PLEON Sampling

From the Pocono Lakes Ecological Observatory Network

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I. Summary 2024: Twin and Walker Lakes at a Glance

PLEON partnered with TWCWC to monitor Big Twin, Little Twin, and Walker lakes three times in 2024 with PLEON on-site in July.

Table 1: Summary of 2024 monitoring.

	Variables Monitored	Crew
15 June 16 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
19 July* 22July	• 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) Olive Stern, Jayson Genao (PLEON interns)
16 Aug 18 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

** Samples for epilimnetic chlorophyll, TN, and TP collected by TWCWC on 19 July 2024. Profile and plankton data collected by PLEON on 22 July 2024*

Table 2: Summary of Big Twin Lake in 2024

	15 June	19 & 22 July**	16 Aug
Thermally stratified?	YES	YES	YES
Epilimnion depth (m)	4	3	5
Metalimnion depth (m)	8	7	9
Secchi depth (m)	1.9	2.25	2.6
Vertical extinction coefficient (k)	—	0.93	—
$Z_{10\%}$ (m)	—	2.46	—
$Z_{1\%}$ (m)	—	4.93	—
Mean hypolimnetic DO (mg/L)	1.07	0.073	0.70
Epilimnetic chlorophyll (µg/L)	1.52	1.24	2.50
Epilimnietic TN (mg/L)	0.259	0.309	0.234
Epilimnetic TP (µg/L)	9.98	6.95	7.08
TSI_{secchi}	50.8	48.3	46.2
$TSI_{chlorophyll}$	34.7	32.7	39.6
TSI_{TP}	37.3	32.1	32.4
Trophic classification*	OLIGOTROPHIC	OLIGOTROPHIC	OLIGOTROPHIC

**according to $TSI_{chlorophyll}$ **Samples for epilimnetic chlorophyll, TN, and TP collected by TWCWC on 19 July 2024. Profile and plankton data collected by PLEON on 22 July 2024.*

Table 3: Summary of Little Twin Lake in 2024

	15 June	19 & 22 July**	16 Aug
Thermally stratified?	YES	YES	YES
Epilimnion depth (m)	3	3	4
Metalimnion depth (m)	10	8	9
Secchi depth (m)	3.1	2.5	3.0
Vertical extinction coefficient (k)	—	0.72	—
$Z_{10\%}$ (m)	—	3.18	—
$Z_{1\%}$ (m)	—	6.36	—
Mean hypolimnetic DO (mg/L)	4.45	0.25	3.45
Epilimnetic chlorophyll (µg/L)	1.53	0.42	3.36
Epilimnietic TN (mg/L)	0.253	0.215	0.241
Epilimnetic TP (µg/L)	5.55	4.45	5.75
TSI_{secchi}	43.7	46.8	44.2
$TSI_{chlorophyll}$	34.7	22.0	42.5
TSI_{TP}	28.9	25.7	29.4
Trophic classification*	OLIGOTROPHIC	OLIGOTROPHIC	MESOTROPHIC

*according to $TSI_{chlorophyll}$ ** Samples for epilimnetic chlorophyll, TN, and TP collected by TWCWC on 19 July 2024. Profile and plankton data collected by PLEON on 22 July 2024*

Table 4: Summary of Walker Lake in 2024

	16 June	22 July	18 Aug
Thermally stratified?	YES	YES	YES
Epilimnion depth (m)	2	2.5	2.5
Metalimnion depth (m)	5	6	6
Secchi depth (m)	1.0	1.25	1.2
Vertical extinction coefficient (k)	—	1.49	—
$Z_{10\%}$ (m)	—	1.55	—
$Z_{1\%}$ (m)	—	3.10	—
Mean hypolimnetic DO (mg/L)	0.74	0.045	0.67
Epilimnetic chlorophyll (µg/L)	2.08	6.31	17.20
Epilimnietic TN (mg/L)	0.594	0.276	0.370
Epilimnetic TP (µg/L)	18.8	21.7	22.1
TSI_{secchi}	60.0	56.8	57.4
$TSI_{chlorophyll}$	37.7	48.7	58.5
TSI_{TP}	46.5	48.5	48.8
Trophic classification*	OLIGOTROPHIC	MESOTROPHIC	EUTROPHIC

** according to $TSI_{chlorophyll}$*

II. Chemical Profiles

A. Temperature

Big Twin was thermally stratified during all 2024 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 21.0 °C (±0.11) during the June sampling, 26.1 °C (±0.28) during the July sampling, and 23.4 °C (±0.29) during the August sampling. The metalimnion, or middle layer of rapid temperature change, extended to 8 m, 7 m, and 9 m during the June, July, and August samplings, respectively.

Little Twin was thermally stratified during all 2024 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 21.6 °C (±0.57) during the June sampling, 26.8 °C (±0.30) during the July sampling, and 24.2 °C (±0.48) during the August sampling. The metalimnion extended to 10 m, 8 m, and 9 m during June, July, and August samplings, respectively.

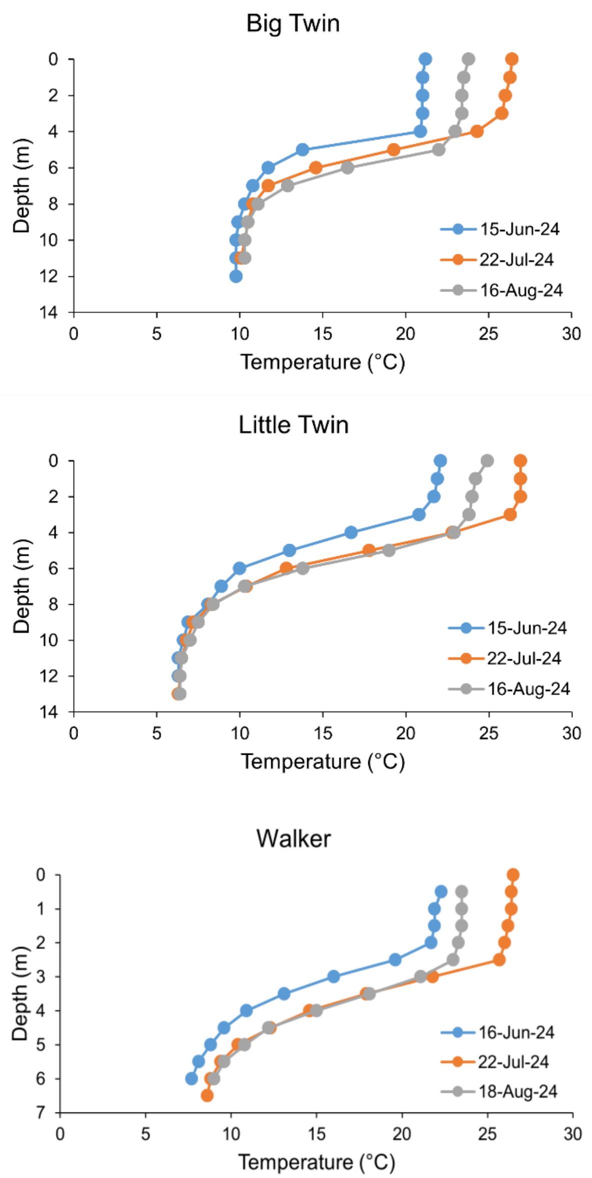


Figure 1: Temperature depth profiles for TWCWC lakes during the summer of 2024. Note scale difference among Y-axes.

Walker was thermally stratified during all 2024 samplings (Figure 1). The epilimnion extended to 2 m, 2.5 m, and 2.5 m during the June, July and August samplings, respectively. The average epilimnetic temperature (± standard deviation) in Walker was 22.0 °C (±0.25) during the June sampling, 26.2 °C (±0.30) during the July sampling, and 23.4 °C (±0.22) during the August sampling. The metalimnion extended to 5 m, 6 m, and 6 m during June, July, and August samplings, respectively.

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.

B. Dissolved Oxygen

Big Twin was oxygenated through the epilimnion during all 2024 samplings (Figure 2). Dissolved oxygen concentration (DO) declined through the metalimnion during all samplings. Average DO concentration in the hypolimnion, or deep water, was 1.07 mg/L during the June sampling, 0.07 mg/L during the July sampling, and 0.70 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 9 m, 5 m, and 7 m during the June, July, and August samplings, respectively.

Little Twin was also oxygenated through the epilimnion during all 2024 samplings (Figure 2). The maximum DO occurred in the metalimnion on all dates, with maximum concentrations at 6 m in June and 7 m in July and August. DO concentration declined at depths below these maxima. Average DO in the hypolimnion was 4.45 mg/L during the June sampling, 0.248 mg/L during the July sampling, and 3.45 mg/L during the August sampling. DO did not fall below 2.0 mg/L at any depth in June. Hypoxia occurred at depths of 9 m in July and 13 m in August.

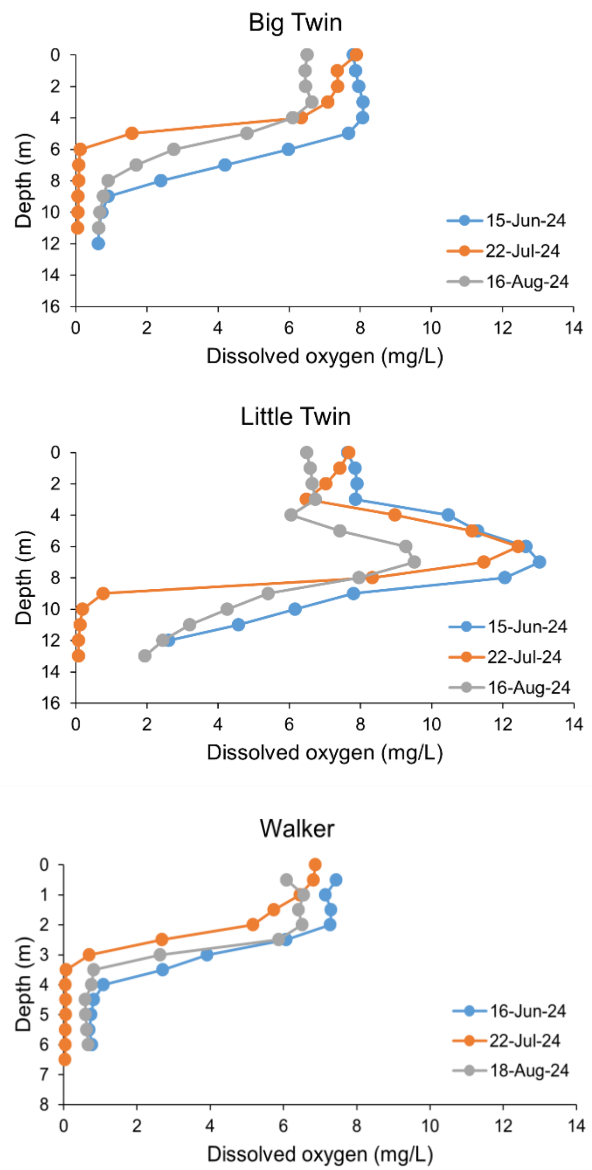


Figure 2: Dissolved oxygen profiles for TWCWC lakes during the summer of 2024. Note scale differences among the Y-axes.

Walker Lake was oxygenated through the epilimnion and DO generally declined through the metalimnion during all 2024 samplings (Figure 2). Average hypolimnetic DO concentration was 0.74 mg/L during the June sampling, 0.045 mg/L during the July sampling, and 0.67 mg/L during the August sampling.

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 byproduct 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 65.4-94.48 µS/cm during the June sampling, from 67.1-91.2 µS/cm during the July sampling, and from 67.2-108 µS/cm during the August sampling. Conductivity in the hypolimnion was greater during the August 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 116.7-134.2 µS/cm during the June sampling, from 88.2-137.3 µS/cm during the July sampling, and from 115.9-151.1 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 59.1-126.3 µS/cm during the June

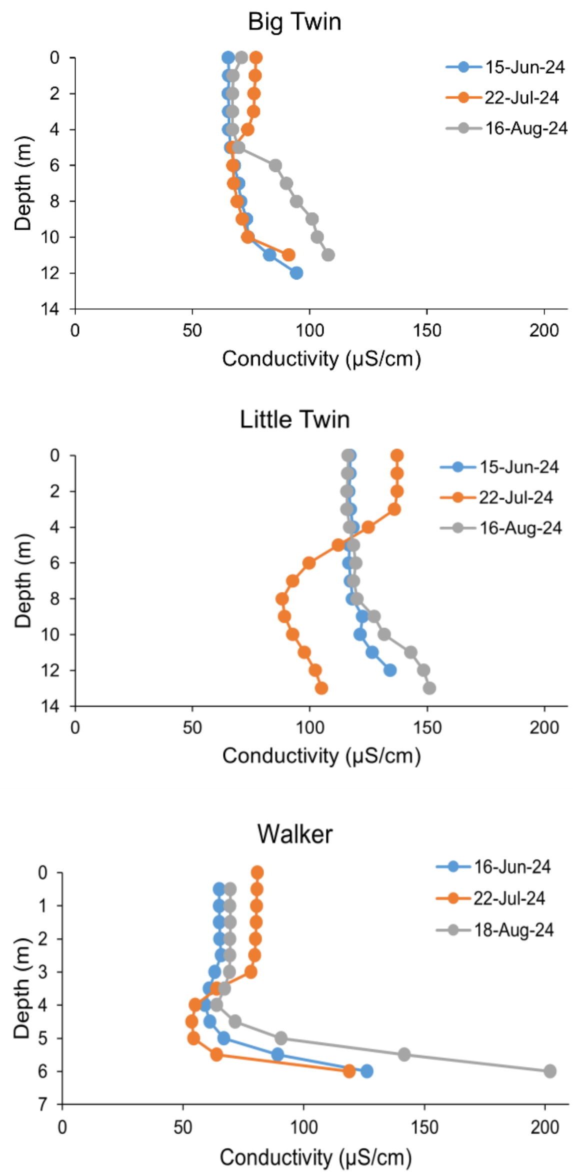


Figure 3: Conductivity depth profiles for TWCWC lakes during the summer of 2024. Note scale differences among the Y-axes.

sampling, from 53.6-143.9 µS/cm during the July sampling, and from 63.9-202.1 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 2024 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 5.58-7.41 during the June sampling, from 6.07-7.34 during the July sampling, and from 6.29-8.07 during the August sampling (Figure 4). pH in Big Twin decreased through the epilimnion, then decreased rapidly through the metalimnion before increasing through the hypolimnion.

pH in Little Twin ranged from 6.43-7.67 during the June sampling, from 6.45-7.28 during the July sampling, and from 6.58-7.58 during the August sampling (Figure 4). Little Twin pH was more stable than Big Twin and Walker. In July and August pH was stable in the epilimnion, decreased in the metalimnion, then increased slightly in the hypolimnion. The June pH profile differed, as pH rose in the metalimnion before decreasing more drastically down through the profile to the sediments.

pH in Walker ranged from 5.75-7.26 during the June sampling, from 5.93-7.40 during the July sampling, and from 5.80-7.56 during the August sampling (Figure 4). pH in Walker, like Big Twin, was lowest in the metalimnion near 4 m depth on all three sampling dates. pH decreased through the epilimnion, decreased more rapidly through the metalimnion, and increased through the hypolimnion.

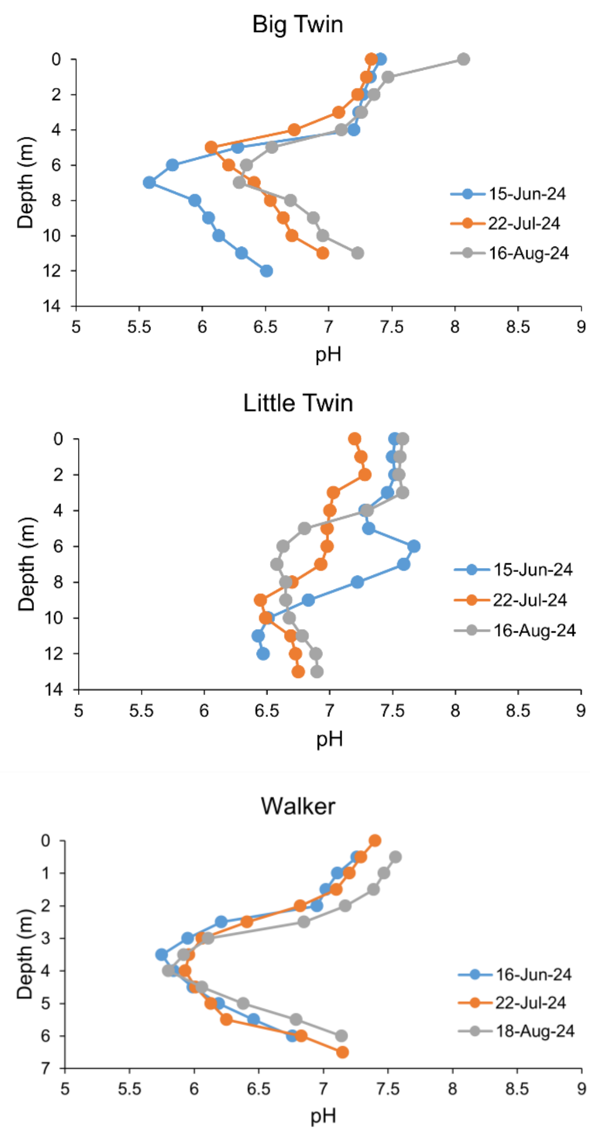


Figure 4: pH depth profiles for TWCWC lakes during the summer of 2024. Note scale differences among Y-axes.

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 Twin and Walker lakes tended toward the acidic end of this range with pH values in the middle depths falling slightly below this range. Several factors affect water pH, including geology, precipitation, runoff, point-source inputs, and carbon dioxide. Carbon dioxide, a byproduct 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]:

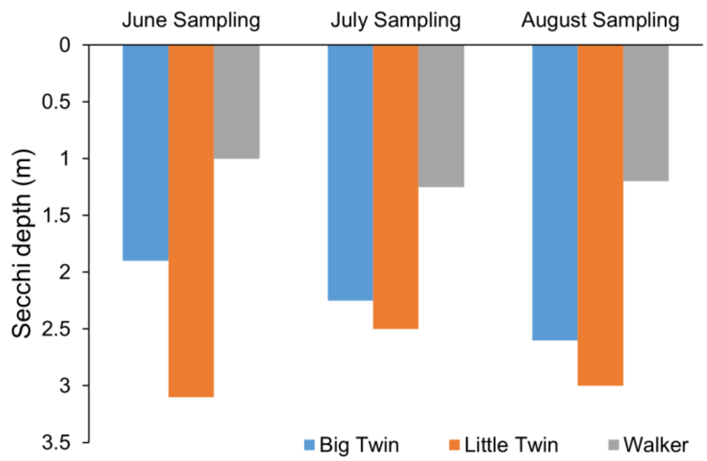


Figure 5: Secchi Depth for TWCWC lakes during summer of 2024. Sampling dates are summarized in Table 1.

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

Secchi depth in Big Twin was 1.9 m, 2.25 m, and 2.6 m during the June, July, and August samplings, respectively (Figure 5). TSI_{Secchi} of Big Twin across these samplings was 50.8, 48.3, and 46.2, respectively, classifying Big Twin as eutrophic for June and mesotrophic for the July and August samplings (Table 5).

Secchi depth in Little Twin was 3.1 m, 2.5 m, and 3.0 m during the June, July, and August sampling, respectively (Figure 5). TSI_{Secchi} of Little Twin across these samplings was 43.7, 46.8, and 44.2, respectively, classifying Little Twin as mesotrophic during all sampling dates (Table 5).

Walker was generally the least clear TWCWC lake on all 2024 sampling dates (Figure 5). Secchi depth in Walker was 1.0 m, 1.25 m, and 1.20 m during the June, July, and

August sampling, respectively. TSI_{Secchi} of Walker across these samplings was 60.0, 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 2024 sampling. Little Twin was the clearest of the TWCWC lakes at this time (k = 0.72, $Z_{10\%}$ = 3.18, $Z_{1\%}$ = 6.36), followed by Big Twin (k = 0.93, $Z_{10\%}$ = 2.46, $Z_{1\%}$ = 4.93), and Walker (k = 1.49, $Z_{10\%}$ = 1.55, $Z_{1\%}$ = 3.10).

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), metalimnion (Big Twin and Little Twin), and composite samples (Walker).

Chla concentration at 0.5 m in Big Twin ranged from 1.24 µg/L to 2.50 µg/L over the 2024 samplings (Figure 6). The greatest epilimnetic chla concentration occurred in August and the lowest concentration was in July. Metalimnion samples were consistently higher in chlorophyll concentration at each sampling date, ranging from 2.76 µg/L to 3.54 µg/L, suggesting that algae were more densely concentrated in the metalimnion.

Chla concentration at 0.5 m in Little Twin ranged from 0.42 µg/L to 3.36 µg/L over the 2024 samplings (Figure 6). Algal abundance in the epilimnion was greatest during the

August sampling and lowest during the July sampling. Metalimnion samples had a greater concentration for all months sampled, ranging from 1.21 µg/L to 10.05 µg/L. This corresponds with the increased DO in the metalimnion.

Walker was the most productive of the TWCWC lakes during the summer of 2024; note the larger scale for the Y-axis for chla concentration (Figure 6). Chla concentration at 0.5 m in Walker ranged from 2.08 µg/L to 17.20 µg/L with chla concentrations increasing over the summer. The chla concentration in the June composite sample (2.04 µg/L) was similar to that of the epilimnion. In July and August, composite samples were higher than those in the epilimnion, with values of 13.21 µg/L and 19.75 µg/L, respectively.

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 34.7, 32.7, and 39.6 during the June, July, and August sampling, respectively, classifying Big Twin as oligotrophic (Table 5).

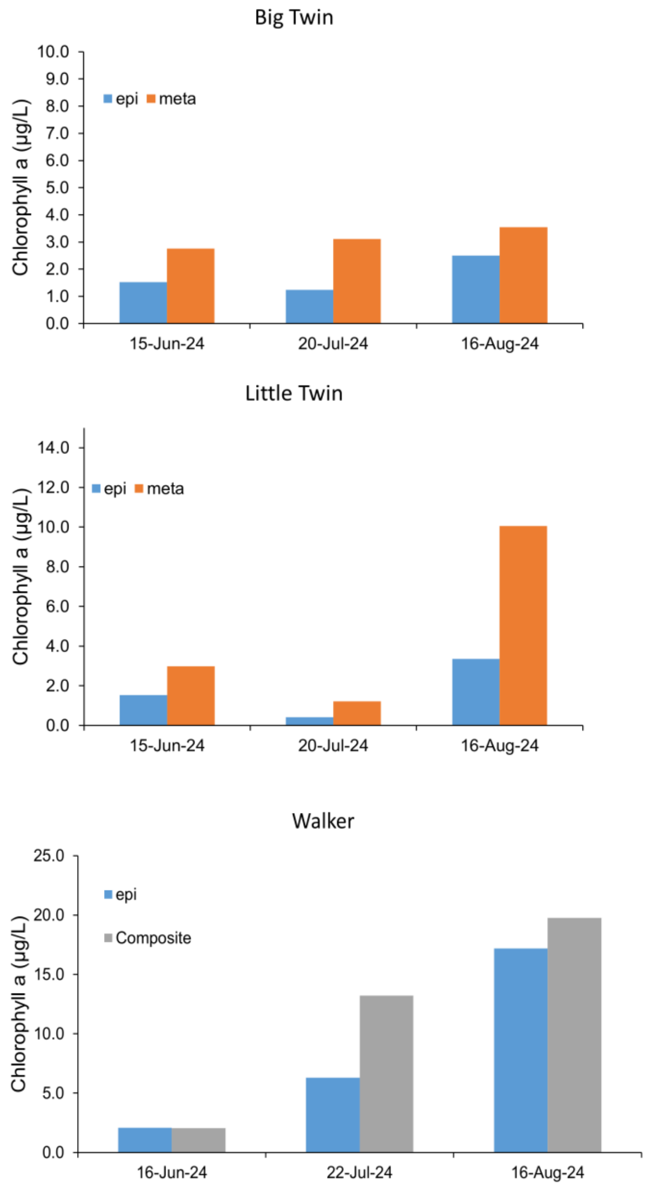


Figure 6: Chlorophyll-a concentrations in TWCWC lakes during 2024. Note scale differences among Y-axes and sampling dates among lakes (summarized in Table 1).

The $TSI_{chlorophyll}$ of Little Twin was 34.8, 22.0, and 42.5 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 37.8, 48.7, and 58.5 during the June, July, and August samplings, respectively. These TSI values classify Walker as oligotrophic in June, mesotrophic in July, and eutrophic in August (Table 5).

V. Nutrient Results

A. Total nitrogen

Total nitrogen concentration (TN) in samples collected from 0.5 m in Big Twin ranged from 0.23 mg/L to 0.31 mg/L, peaking in July (Figure 7). TN was consistently greater in surface samples than deep water samples.

TN in samples collected from 0.5 m in Little Twin ranged from 0.22 mg/L to 0.25 mg/L during 2024 (Figure 7). Hypolimnion samples had greater TN compared to surface samples in Little Twin. TN in hypolimnetic samples was nearly 2x the epilimnetic concentrations in July and August.

TN in samples collected from 0.5 m in Walker ranged from 0.28 mg/L to 0.59 mg/L during the summer of 2024 (Figure 7). TN concentrations were less in composite samples in June and July. The August composite sample had a TN concentration slightly higher than the surface sample.

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].

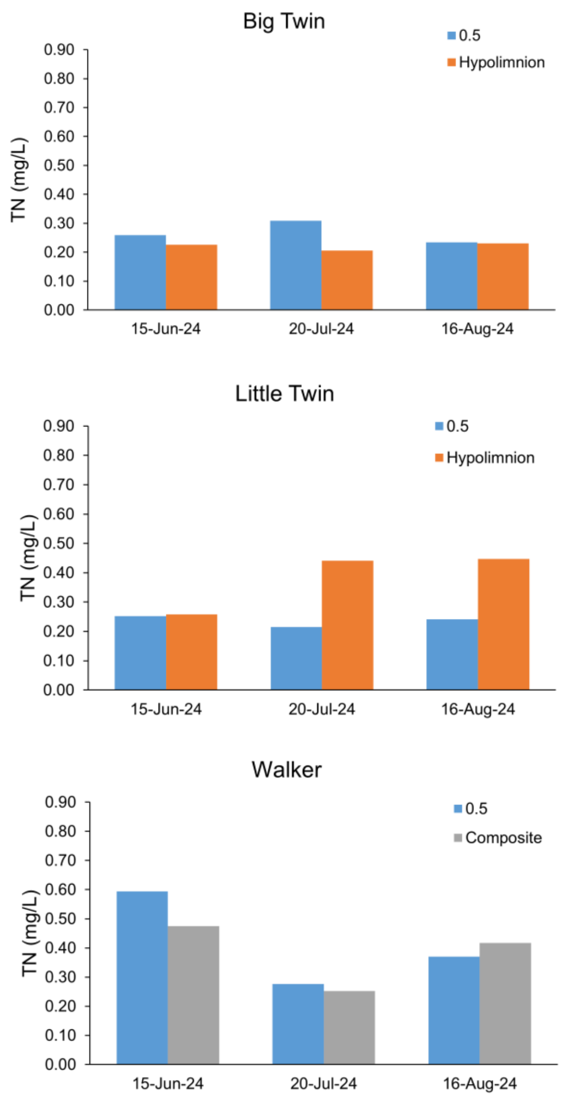


Figure 7: TN concentrations of water samples collected at varying depths for TWCWC lakes for the summer of 2024.

B. Total phosphorus

Total phosphorus concentration (TP) in samples collected from 0.5 m in Big Twin ranged from 6.95 µg/L to 9.98 µg/L during the 2024 samplings (Figure 8). The greatest epilimnetic TP concentration occurred during the June sampling. TP concentrations in the hypolimnion were greater than those of the

0.5 m samples suggesting TP was more concentrated at deeper depths. The highest hypolimnetic TP concentration occurred in August, at 29.88 µg/L.

TP in samples collected from 0.5 m in Little Twin ranged from 4.45 µg/L to 5.75 µg/L across all 2024 samplings (Figure 8). Hypolimnetic TP concentrations were higher than all surface samples. TP in the hypolimnion during the July sampling was 18x higher than that in the epilimnion, at 81.95 µg/L. August hypolimnetic TP was also much greater than that of the 0.5 m sample.

The epilimnetic TP concentrations were highest in Walker in 2024 (Figure 8). TP in samples from 0.5 m in Walker ranged from 18.8 µg/L to 21.70 µg/L during the 2024 samplings. TP in the composite sample were lower than the 0.5 m samples in June and July, but slightly higher in August.

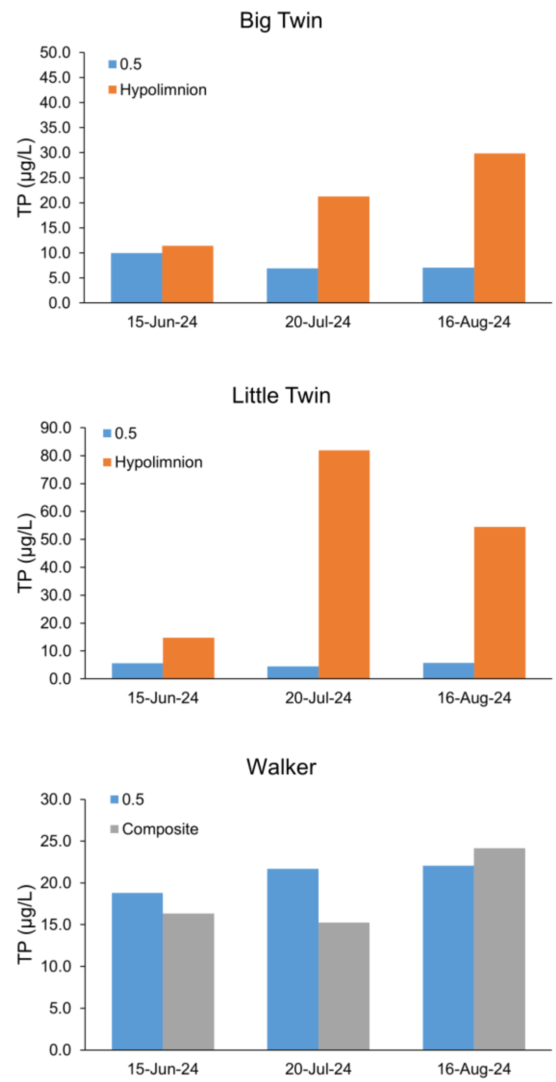


Figure 8: TP concentrations at varying depths for TWCWC lakes during the summer of 2024. Sampling dates are summarized in Table 1.

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 all three lakes were below the 25 µg/L threshold for nutrient pollution suggested by Penn State Extension[1] during 2024 samplings. Hypolimnetic TP in the Twin lakes was closer to this threshold, exceeding it in July in Little Twin and August in both Little Twin and Big Twin.

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 hypolimnetic samples exceeding that of surface samples in TWCWC lakes.

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

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

TSI_{TP} of Big Twin was 37.3, 32.1, and 32.4 during the June, July, and August samplings, respectively. TSI_{TP} classified Big Twin as oligotrophic during all 2024 samplings (Table 5).

TSI_{TP} of Little Twin was 28.9, 25.7, and 29.4 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 46.5, 48.5, and 48.8 during the June, July, and August sampling respectively. TSI_{TP} classified Walker as mesotrophic during all 2024 samplings (Table 5).

C. Dissolved organic carbon

Dissolved organic carbon concentration (DOC) samples were collected at 0.5 m depth from each lake in June, July, and August. Big Twin was 3.71 mg/L on June 15, 3.71 mg/L on July 19, and 3.73 mg/L on August 16. Little Twin DOC was 3.40 mg/L on June 15, 2.95 mg/L on July 19, and 3.02 mg/L on August 16. DOC in Walker was 3.85 mg/L on June 16, 3.80 mg/L on July 22, and 3.70 mg/L on August 18.

The pool of DOC in lakes includes soluble organic compounds that wash in from the watershed, byproducts 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. DOC impacts water clarity, which can further impact temperature stratification, dissolved oxygen levels, and the phytoplankton community[5].

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 22 July 2024. Walker was not sampled for zooplankton.

Zooplankton numbers in both lakes were dominated by rotifers, which made up 59% and 82% 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 a lesser percentage of total zooplankton biomass (8% biomass in Big Twin, 9% biomass in Little Twin).

Copepods made up 34% and 83% 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 July 22, 2024 (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	**0**	**0%**	**0**	**0%**	**0**	**0%**	**0**	**0%**
Ciliophora	0		0		0		0	
ROTIFERA	**117**	**59%**	**16**	**8%**	**101**	**82%**	**20**	**9%**
Asplanchna	2		5		2		12	
Brachionus								
Conochilus	22		1		33		1	
Filinia	0		0		0		0	
Hexarthra	5		0		1		0	
Keratella	54		5		46		4	
Lepadella	0		0		0		0	
Polyarthra	25		2		9		1	
Trichocerca	9		3		9		2	
COPEPODA	**68**	**34%**	**174**	**83%**	**23**	**19%**	**58**	**27%**
Copepoda-Cyclopoida	10		20		9		20	
Cyclops	6		14		7		17	
Mesocyclops	4		5		2		2	
Copepoda-Calanoida	0		0		0		0	
Other Copepoda-Naupli	58		154		15		39	
CLADOCERA	**13**	**6%**	**20**	**10%**	**13**	**11%**	**55**	**25%**
Bosmina	10		9		2		2	
Ceriodaphnia	3		7		9		32	
Daphnia catawba	0		0		0		2	
Holopedium	0		4		2		19	
OTHER ZOOPLANKTON	**0**	**0%**	**0**	**0%**	2	**0%**	**83**	**39%**
TOTAL	**198**		**211**		**123**		**217**	

Table 6) and Cladocerans were found in relatively low densities but accounted for 10% and 64% 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 11.5 and 13.5, respectively and average diversity (Shannon-Wiener Index) was 0.82 and 0.85, 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 22 July 2024.

Cyanophyta (cyanobacteria) were the numerically dominant group in both Big Twin (43% phytoplankton density) and Little Twin Lake (86% phytoplankton density). Chlorophyta were the second-most dominant group in Big Twin, making of 35% of phytoplankton density, while Bacillariophyta made up 15%. In Little Twin, Chlorophyta was the second most dominant group making up 7.7% of phytoplankton density. Other groups of algae were in low abundance (Table 7).

The cyanobacteria, community in the Twin Lakes was composed of genera capable of producing toxins that can be harmful to humans and pets. In Big Twin, the genera *Dolichospermum, Limnothrix, Woronichinia*, and *Spirulina* were identified (listed in order of greatest abundant to least abundant). The Little Twin community consisted of *Limnothrix, Aphanizomenon*, and *Dolichospermum*.

Average phytoplankton taxonomic richness in Big Twin was 20.5 and average diversity (measured using the Shannon-Wiener Index) was 0.89. Planktonic richness in Little Twin was 19.5 and diversity was 0.42.

Table 7: Phytoplankton community in the Twin Lakes on July 22, 2024 (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	1124	15%	831	17%	114	0.8%	273	7%
Centric Diatoms	64		43		13		15	
Araphid Pennate Diatoms	1016		751		102		258	
CHLOROPHYTA	2584	35%	794	17%	1048	7.7%	658	17%
Coccoid/Colonial Chlorophytes	0		0		241		41	
Filamentous Chlorophytes	2356		471		279		56	
Desmids	229		323		476		541	
CHRYSOPHYTA	140	2%	232	5%	648	5%	1775	45%
Flagellated Classic Chrysophytes	140		232		648		1775	
CRYPTOPHYTA	267	4%	1798	38%	38	0.3%	25	0.6%
CYANOPHYTA	3239	43%	418	9%	11779	86%	234	6%
Filamentous Nitrogen Fixers	2032		406		857		125	
Filamentous Non-Nitrogen Fixers	826		8		10922		109	
EUGLENOPHYTA	64	1%	8	0%	0	0.0%	109	2.7%
TOTAL	7449		4790		13672		3985	

VII. 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 2014-2024.

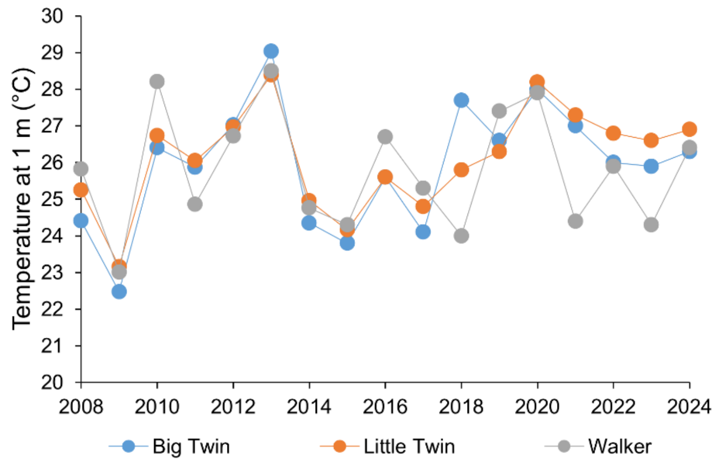


Figure 9: July temperature at 1 m in TWCWC lakes from 2008 to 2024.

TWCWC lakes were generally stratified in the summer months (June, July, August) from 2014-2024. Surface temperature in TWCWC lakes, while variable, have generally increased over this period, though not with statistical significance (Figure 9).

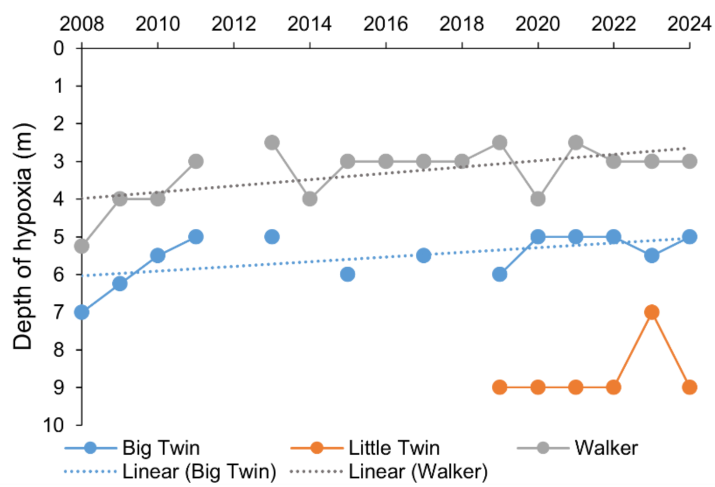


Figure 10: Depth of hypoxia in July for TWCWC lakes from 2008 to 2024.

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 trendlines for Big Twin and Walker data indicate 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.

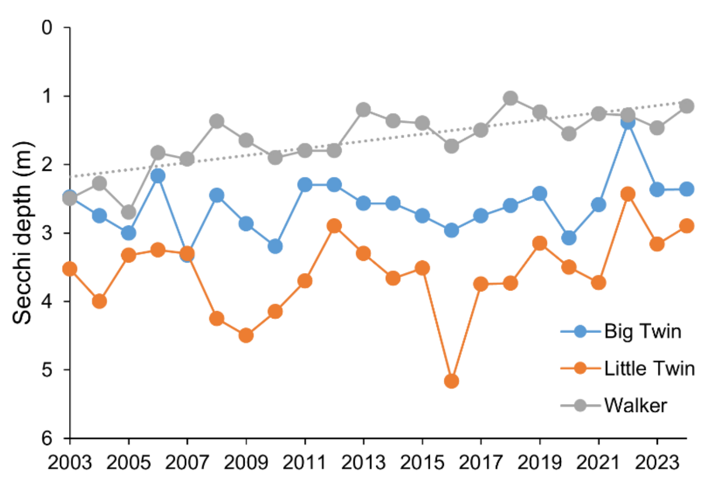


Figure 11: Average summer (June, July, August) Secchi depth for TWCWC lakes from 2003 to 2024.

Conductivity in Little Twin was consistently greater than that of Big Twin and Walker across the dataset, with an average conductivity of 136.0 µS/cm compared to 80.8 µS/cm and 80.9 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 6.62 compared to 7.00 and 7.12 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 1.6 m (averages include all readings in June, July, and August from 2003-2024;

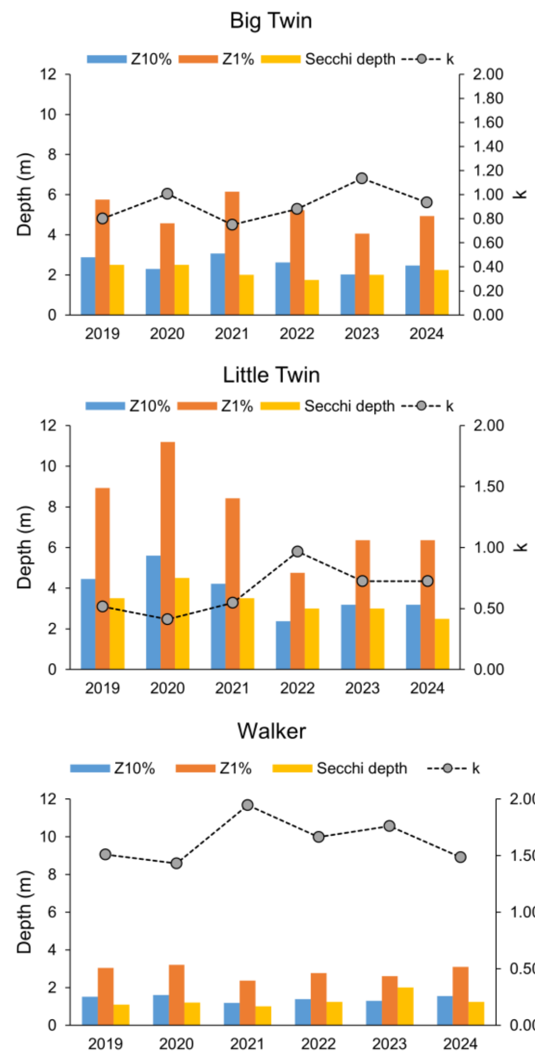


Figure 12: Light attenuation in TWCWC lakes from 2019-2024. Measurements made in July.

Figure 11). Secchi depth in Walker decreased over the 20-year dataset (linear regression, $r^2 = 0.64$, $p < 0.01$; Figure 11).

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).

D. Chlorophyll *a* over time

Chla concentration has been measured in TWCWC lakes since 2003. Over this time, average summer (June, July, August) chla concentration at 0.5 m has ranged from 1.75 µg/L to 10.2 µg/L in Big Twin, from 0.71 µg/L to 8.2 µg/L in Little Twin, and from 2.3 µg/L to 19.3 µg/L in Walker (Figure 13; top panel). Chla concentrations generally declined over time, with a statistically significant decline in Big Twin (linear regression, $r^2 = 0.22$, $p = 0.03$) and Little Twin (linear regression, $r^2 = 0.60$, $p = <0.001$). Chla concentrations in Walker over time were much more variable.

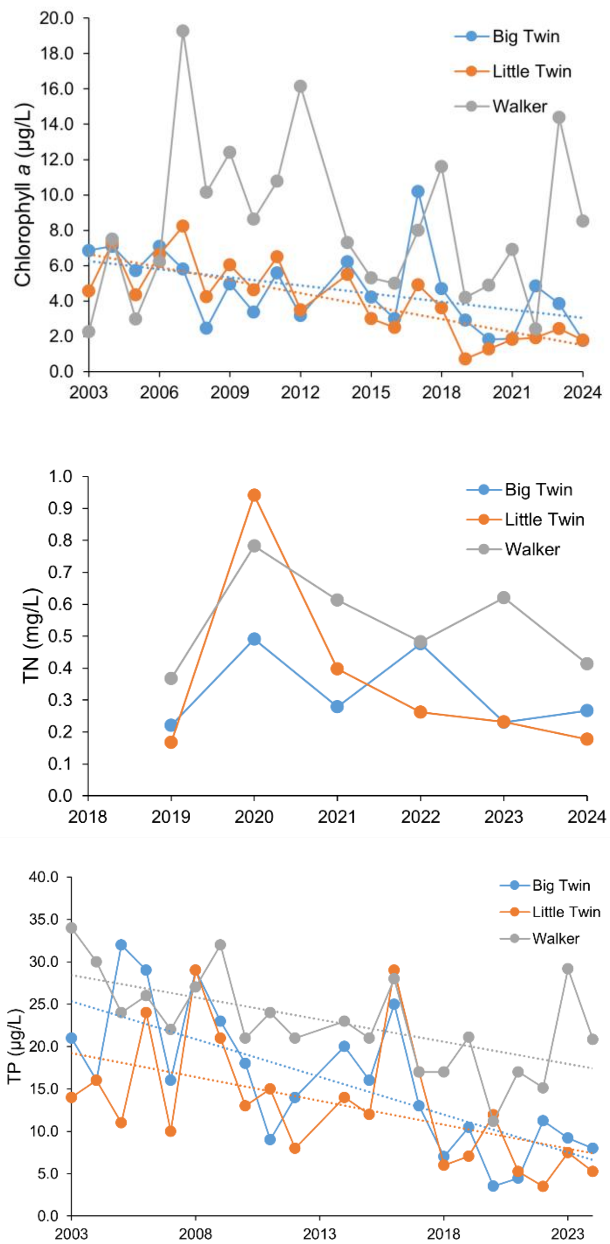


Figure 13: Average epilimnetic chla, TN, and TP in TWCWC lakes from 2003-2024. Dashed lines show significant trends over time.

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; middle panel). The greatest TN occurred in 2020 in all three lakes. TN concentration declined steadily in Little Twin from 2020 to the current year. Concentration of TN was more variable in Big Twin and Walker.

TP has been measured in TWCWC lakes since 2003. Average summer (June, July, August) TP measured in samples collected from 0.5 m ranged from 3.5 µg/L to 32.0

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 34.0 µg/L in Walker (Figure 13; bottom panel). Summer TP generally declined over the 8-year period in all lakes. This decline is statistically significant in Big Twin (linear regression, $r^2 = 0.52$, $p = <0.001$), Little Twin (linear regression, $r^2 = 0.26$, $p = 0.02$), and Walker (linear regression, $r^2 = 0.36$, $p = 0.004$). TP in Walker increased in 2023 and remained high relative to historical concentrations in 2024.

DOC has been measured in the epilimnion of all three lakes from 2020 to 2024. DOC was fairly steadily across the data set for Big Twin and Little Twin (Figure 14). Big Twin had a maximum summer average epilimnetic DOC concentration of 4.53 mg/L in 2022. Little Twin's maximum summer average DOC was 3.66 mg/L in 2023. Walker DOC concentrations were more variable with a decreased from 2020 to 2021 and 2022, followed by an increase in 2023. This 2023 summer average DOC was the highest obtained in Walker, at 5.11 mg/L. Walker had the highest DOC in most years, while Little Twin had the lowest DOC across all years.

F. Trophic status over time

Big Twin and Walker were generally mesotrophic since 2014 and Little Twin was generally oligo-mesotrophic (Figure 15). TSI_{Secchi} was typically greater than $TSI_{chlorophyll}$ and TSI_{TP} in all three lakes, particularly since 2018 when $TSI_{chlorophyll}$ and TSI_{TP} began declining.

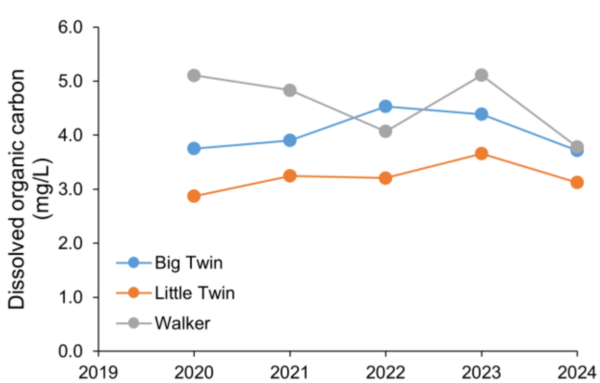


Figure 154: Average summer epilimnetic DOC concentration in TWTWC lakes, from 2020 to 2024.

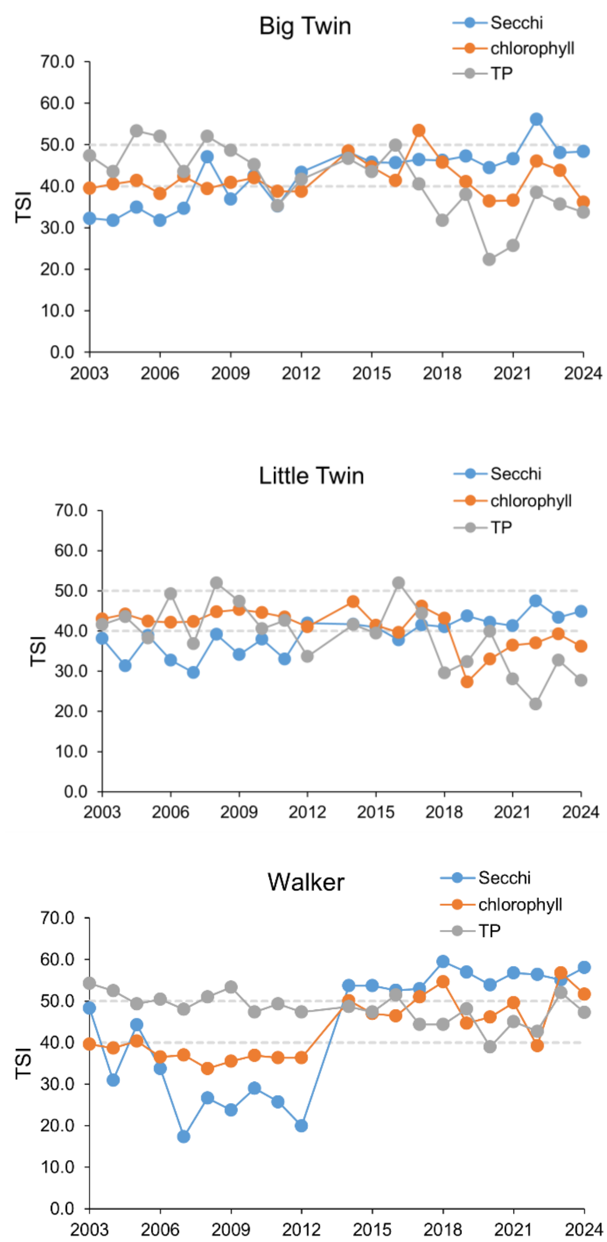


Figure 14: Average summer TSI in TWCWC lakes from 2003-2024 according to Secchi depth, chla, and TP. Horizontal lines show thresholds for oligotrophy (<40), mesotrophy (40-50), and eutrophy (>50).

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 6-year dataset (Figure 16). 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 6-year dataset (Figure 16). Zooplankton diversity ranged from 0.67-0.84 in Big Twin and from 0.63-0.95 in Little Twin. Average zooplankton length ranged from 0.12 mm to 0.21 mm in Big Twin and from 0.15 mm to 0.27 mm in Little Twin.

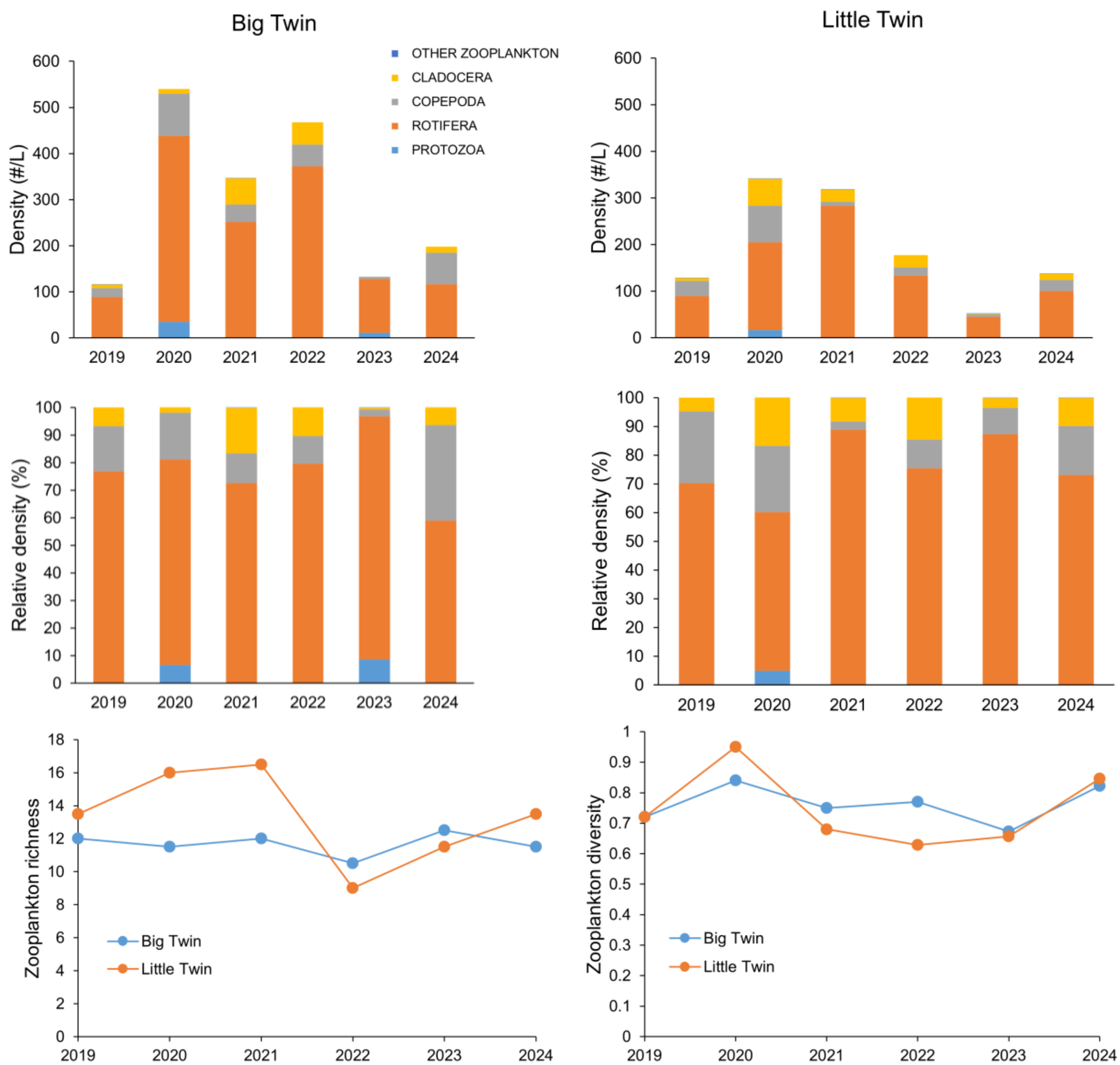


Figure 16: Zooplankton communities in the Twin Lakes from 2019-2024. Samples were collected in July of each year. Bars and symbols are averages of two replicates.

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. Density has since decreased in both lakes, but Cyanophyta still make up a large percentage of phytoplankton density and were the dominant form in both lakes in 2024.

Phytoplankton diversity in both Twin lakes declined from 2020 to 2022 (Figure 17). Diversity in Big Twin Lake increased from 2022 to 2023 with Chlorophyta as the most dominant group. In 2024, Cyanophyta was dominant, and diversity remained high. More

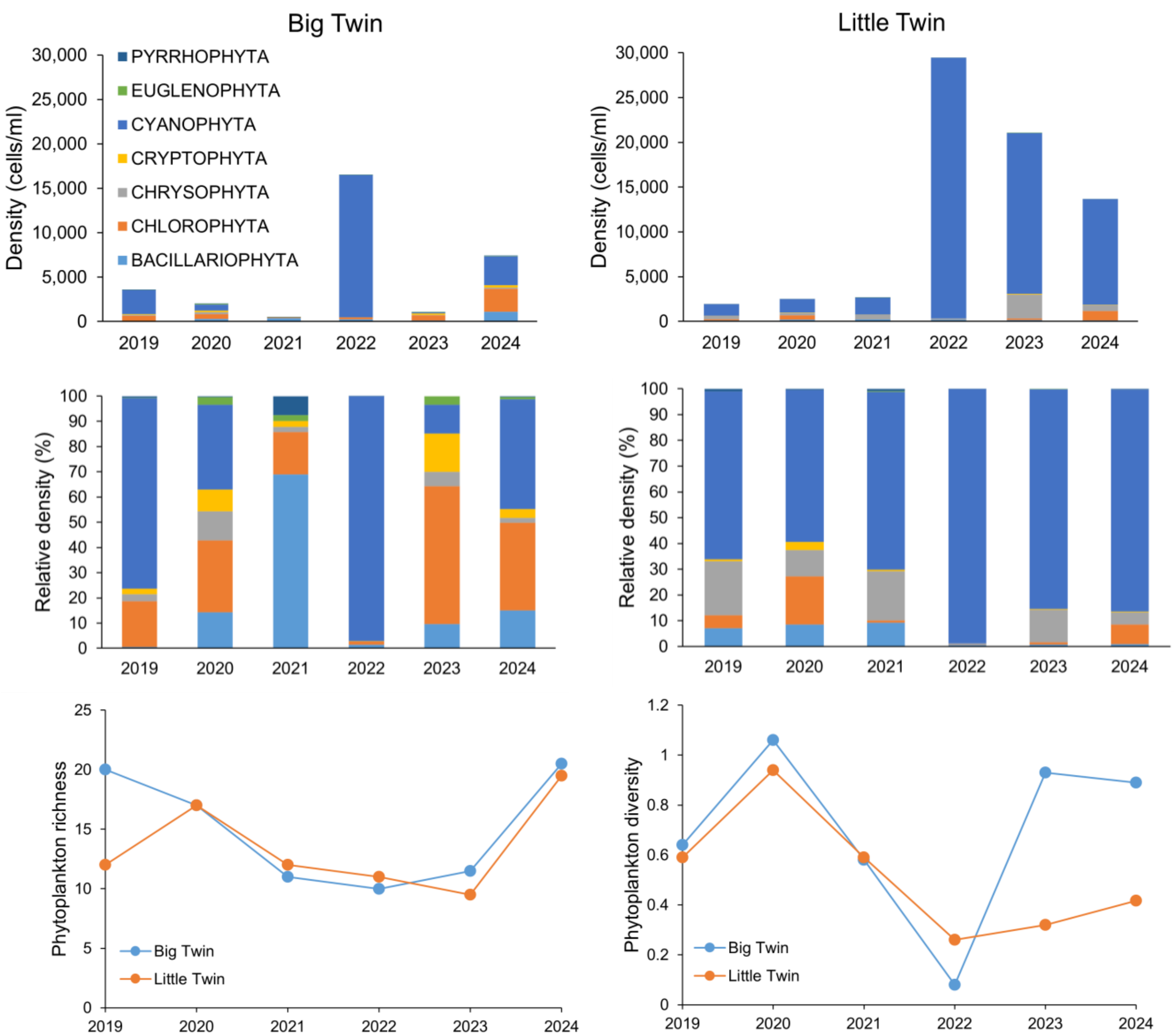


Figure 17: Phytoplankton communities in the Twin Lakes from 2019-2024. Samples were collected in July during each year. Bars and symbols are averages of two replicates.

years of sampling are needed to determine if this shift is due to variation or will become more “normal” for Big Twin Lake.

I. Cyanobacteria and cyanotoxins over time

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.

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. Visible blooms have been observed in Big Twin Lake and PTOX genera have been observed in screens of both Twin Lakes (including *Dolichospermum*, *Aphanizomenon*, and *Chrysosporum*). PLEON (and collaborations) has not observed visible cyanobacteria blooms in Walker, but PTOX screens have contained potentially toxigenic taxa (including *Aphanizomenon* and *Chrysosporum*). Detailed descriptions of PTOX screen results have been presented previously. In-depth phytoplankton community analysis has been conducted on samples collected from the Twin Lakes since 2019. Phytoplankton analyses have indicated the presence of potentially toxigenic taxa in 2023 and 2024, which included the genera *Aphanizomenon*, *Dolichospermum, Limnothrix, Spirulina*, and *Woronichinia*.

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

A. Description of PLEON Lakes

The PLEON dataset consists of 33 lakes in Pike, Wayne, Monroe, Lackawanna, and Schuylkill Counties. Lakes range in surface area, shoreline, and depth (Figure 18). The 33 lakes sampled by PLEON have an average depth of 7.6 m; Big Twin and Little Twin are almost twice the average depth, while Walker is about a meter deeper than the PLEON average.

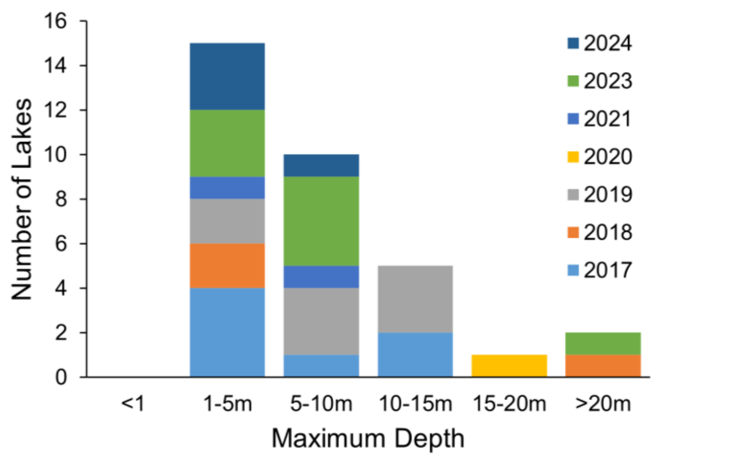


Figure 18: Maximum depth of PLEON lakes. Years refer to the first PLEON sampling year. Not all lakes are sampled every year.

B. Water transparency

PLEON recorded Secchi depth at least once during the summer months (June, July, August) in 19 of the 33 lakes during 2024. The average summer Secchi depth in these lakes ranged from 0.95 m to 4.80 m with an average of 2.37 m (Figure 19). The average summer Secchi depth for Big Twin and Little Twin was close to the PLEON average, while the average Secchi depth for Walker was less than half of the PLEON average depth.

C. Lake productivity

Lake productivity, measured by chla concentration at 0.5 m depth, was assessed in 19 PLEON lakes during the summer months (June, July, August) in 2024. Average summer Chla concentration in these lakes ranged from 0.90 µg/L to 19.99 µg/L with an average of 5.72 µg/L (Figure 19). Walker exceeded this average concentration by about 3 µg/L. Big Twin and Little Twin had average chla concentrations below the PLEON average; with Little Twin at about half the PLEON average concentration and Big Twin about one-third the PLEON average.

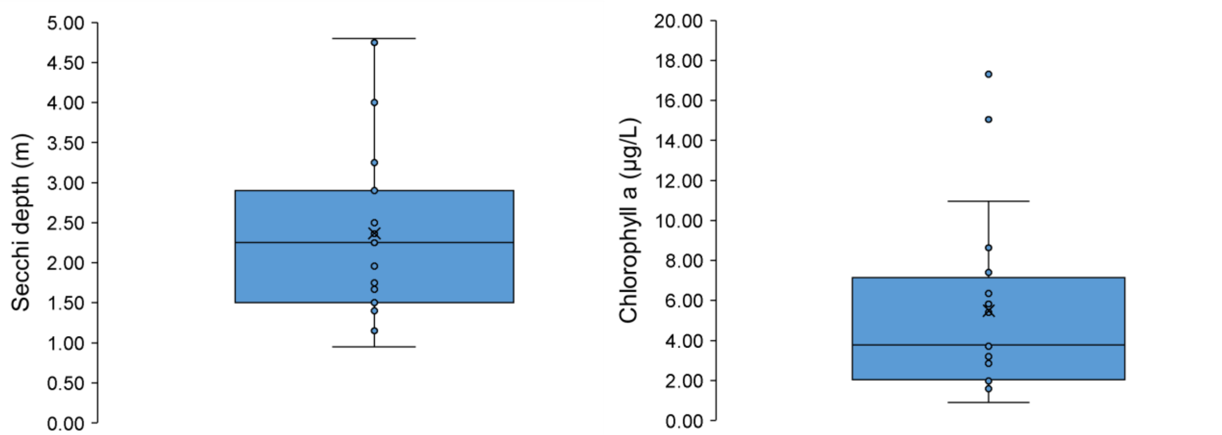


Figure 19: Average summer (June, July, August) Secchi depth (left) and chlorophyll a concentration at 0.5 m (right) across 19 PLEON lakes monitored in 2024. Lines within boxes are medians and X symbols are means. Upper and lower box boundaries denote the 75th and 25th percentile, respectively while upper 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) concentration was quantified at 0.5 m depth for 17 lakes during the summer months of June, July, and August 2024. Average summer TN concentration ranged from 0.174 mg/L to 0.452 mg/L in these lakes, with an average concentration of 0.295 mg/L (Figure 20). The average summer TN for Big Twin (0.267 mg/L) and Little Twin (0.236 mg/L) were less than the PLEON average. Walker was above the PLEON average at 0.413 mg/L.

Total phosphorus (TP) concentration was quantified at 0.5 m depth in 17 PLEON lakes during the summer months (June, July, August) of 2024. Average summer TP concentration ranged from values below detection to 47.4 µg/L, with an average of 17.8 µg/L (Figure 20). The summer average epilimnetic TP concentration in Big Twin and Little Twin were below the PLEON average, at 5.25 µg/L in Little Twin and 8.00 µg/L in Big Twin. The average summer epilimnetic TP concentration in Walker was above the PLEON average, at 20.85 µg/L.

Dissolved organic carbon (DOC) was quantified at 0.5 m depth in 13 PLEON lakes during 2024. Average summer DOC concentrations ranged from 2.07 mg/L to 5.19 mg/L, with an average of 3.73 mg/L (Figure 20). The summer average DOC

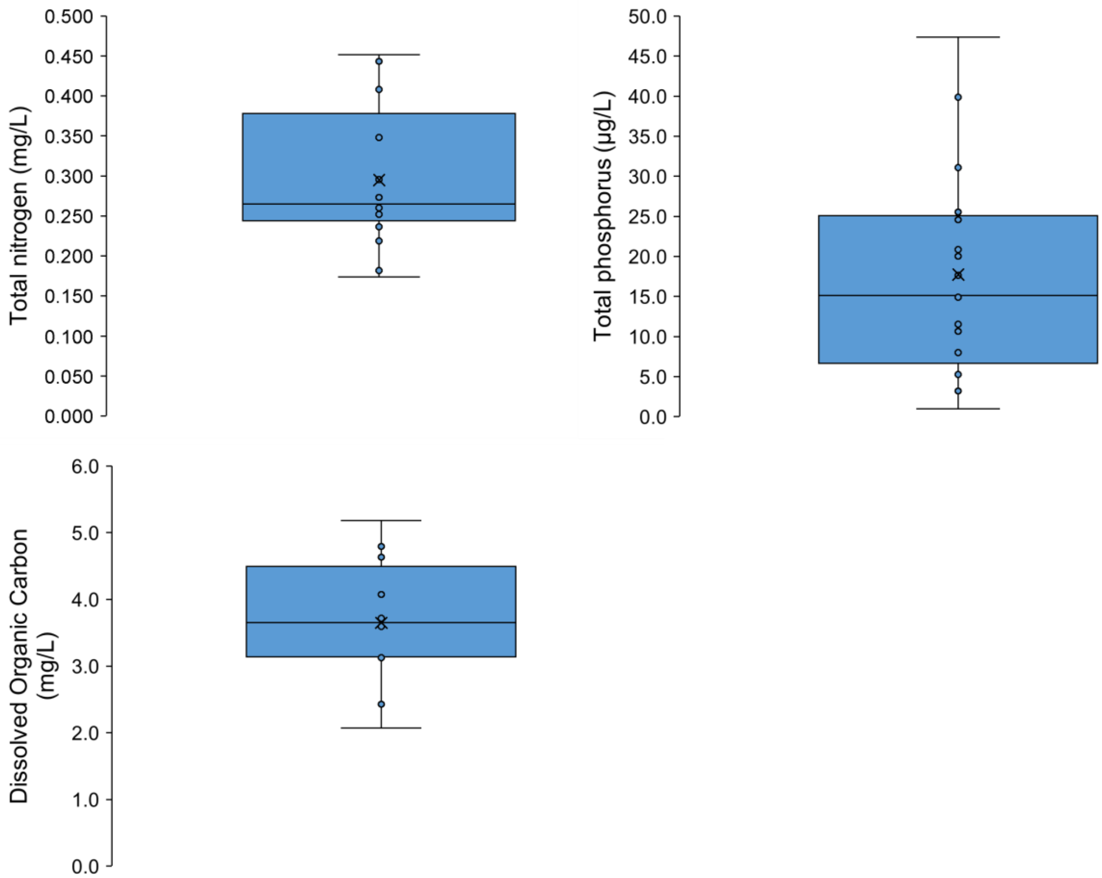


Figure 20: Average summer (J, J, A) TN, TP, and DOC of PLEON lakes monitored in 2024. Lines are medians and X symbols are means. Upper and lower boundaries are the 75th and 25th percentile, respectively. Whiskers show maximum and minimum values. Circles are single measurements or an average if the lake was sampled more than once during the summer. Nutrient concentrations were quantified from 0.5 m depth.

concentration for Little Twin (3.12 mg/L) was below the PLEON average, while average DOC for Big Twin fell right around the PLEON average (3.72 mg/L). Walker had the highest average summer DOC at 3.78 mg/L, which was above the PLEON average.

E. Cyanobacteria

Since 2017, PLEON has collected 295 samples for PTOX screening as a part of its formal monitoring program. These samples were collected from 24 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.

Eleven (possibly 12, some specimens are difficult to identify) PTOX cyanobacteria genera have been found in PLEON samples to date (Figure 21). The most commonly found genera are *Dolichospermum*, followed by *Aphanizomenon* (or *Aphanizomenon*-like). *Chrysosporum*, *Woronichinia*, and *Microcystis* were also common. Eighty-one of the samples (or 27%) did not have PTOX taxa present. Three lakes within the dataset have been consistently free of PTOX taxa but 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.

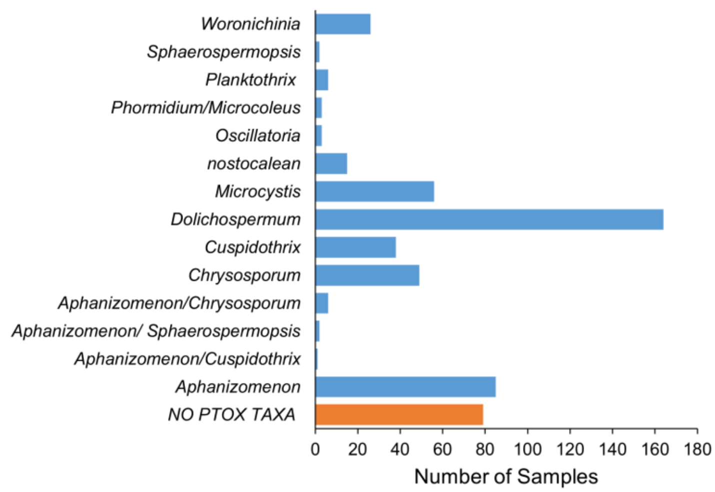


Figure 21: Potentially toxic (PTOX) cyanobacteria genera found in samples collected from PLEON lakes since 2017. PTOX screens were conducted by Greenwater Laboratories.

Based on the results of the PTOX screens, Greenwater Laboratories has recommended quantifying microcystin/nodularin concentration in 29% of the PLEON samples and quantifying cylindrospermopsin, anatoxin-a, and/or saxitoxin concentration in 22% of the samples. Cyanotoxin quantification is an opt-in service; to date, between 64% 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 8). Microcystin/nodularins are hepatotoxins, cylindrospermopsin is a hepatotoxin and a nephrotoxin, and saxitoxin is a potent neurotoxin[6]. 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[7]. 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[8]. 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. Toxin testing was not completed in 2024.

Table 8: Samples tested for cyanotoxins from PLEON lakes since 2017. Cyanotoxin analyses were conducted by Greenwater Laboratories.

Toxin	# samples recommended for testing	# tested	# ≥ MDL*	Mean concentration (ng/mL)	Range (ng/mL)
microcystins/nodularins	86	66	20	9.44	0.16-129
cylindrospermopsin	64	41	1	0.07	-
anatoxin-a	64	44	0	-	-
saxitoxin	64	45	5	0.40	0.15-0.73
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 2024 monitoring program should be highlighted:

1. Algae increased over the summer. TP availability showed a subsequent increase.

Chlorophyll *a* concentration, a proxy for the amount of algae, increased over the summer in all three lakes, with maximum chla concentrations in August. Phosphorus concentrations increased through the summer months in Big Twin and were fairly consistent in Walker. Little Twin TP concentrations increased greatly from June to July and decreasing slightly in August. Availability of phosphorus has correlated with chla concentration in the past in all three 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. Both Little Twin and Big Twin had higher TP concentrations in hypolimnetic samples than epilimnetic samples throughout the summer. However, the overall contribution of internal phosphorus loading relative to other potential sources of phosphorus in these lakes is unknown.

Patterns and implications of deep-water TP in Walker cannot be determined with the current data. TP in composite samples does not represent nutrient conditions in the hypolimnion (the site of potential nutrient regeneration) but instead represent a mix of epilimnetic and metalimnetic conditions. The anoxic conditions of the hypolimnion suggest that potential for nutrient regeneration exists in Walker. However, discrete hypolimnetic samples are needed to better evaluate deepwater nutrient availability in this lake.

2. Correlations between water clarity, algal abundance, and phosphorus availability over the long-term dataset remain weak.

The coinciding changes in water clarity, algal abundance, and phosphorus availability observed over the past several years in the Twin lakes (as described above) suggest that these variables are affecting each other (increases in phosphorus may 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 are not supported (Figure 22). Average summer Secchi depth was not significantly correlated with average summer chlorophyll a concentration in any of the TWCWC lakes (correlation coefficient r ≤ 0.24, suggesting <24% of the variation in one variable responds to the variance in the second variable). Average chlorophyll a concentration also did not respond strongly to TP concentration in any of the three lakes (Figure 22).

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.

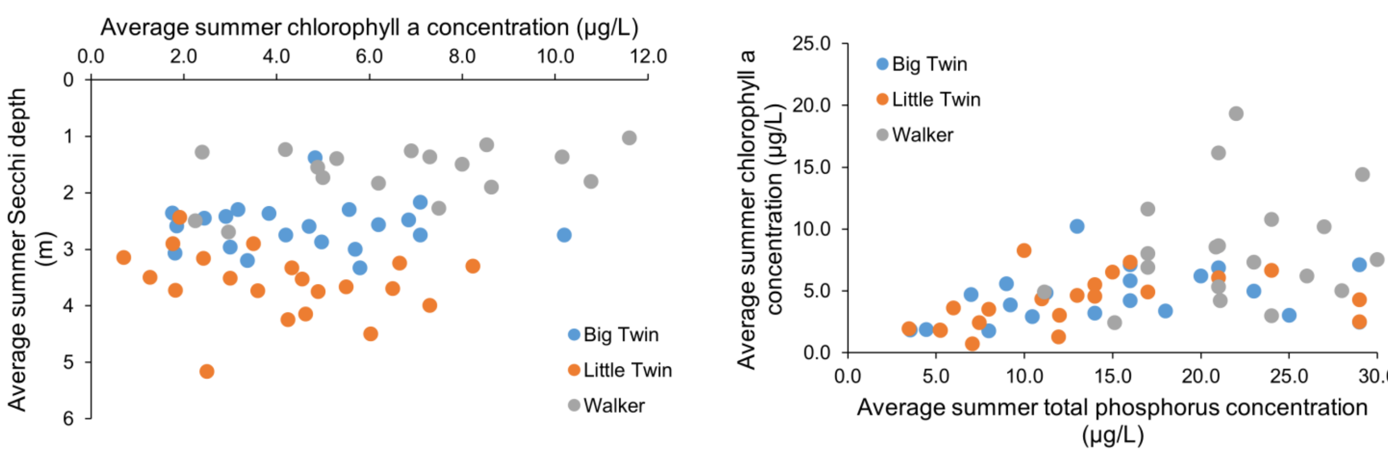


Figure 18: Correlations between average summer Secchi depth and average summer chlorophyll a and between average summer chlorophyll a and average summer TP in TWCWC lakes over the long-term dataset.

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 ($r^2 = 0.64$). 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. Dissolved organic carbon data has only been collected since 2020. DOC has been variable over the 5-year data set, with a maximum of 5.11 mg/L in 2023 and a minimum of 3.78 mg/L in 2024. More data collection could determine if a relationship exists between water clarity and DOC.

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

There are several lines of evidence suggesting reasons for concern:

1. Potentially toxigenic (PTOX) cyanobacteria have been detected in TWCWC lakes in 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 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.
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.

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 2024 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

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 9:1 acetone:water solution. The extraction took place over 18 hours at -20°C. Chlorophyll concentration of the extractant was determined via fluorometry (Turner Designs 10AU fluorometer) and corrected for phaeophytin 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 (EPA methods 353.2 and 365.1, respectively).

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}).

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 Darrin Freshwater Institute at Rensselaer Polytechnic Institute.

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.

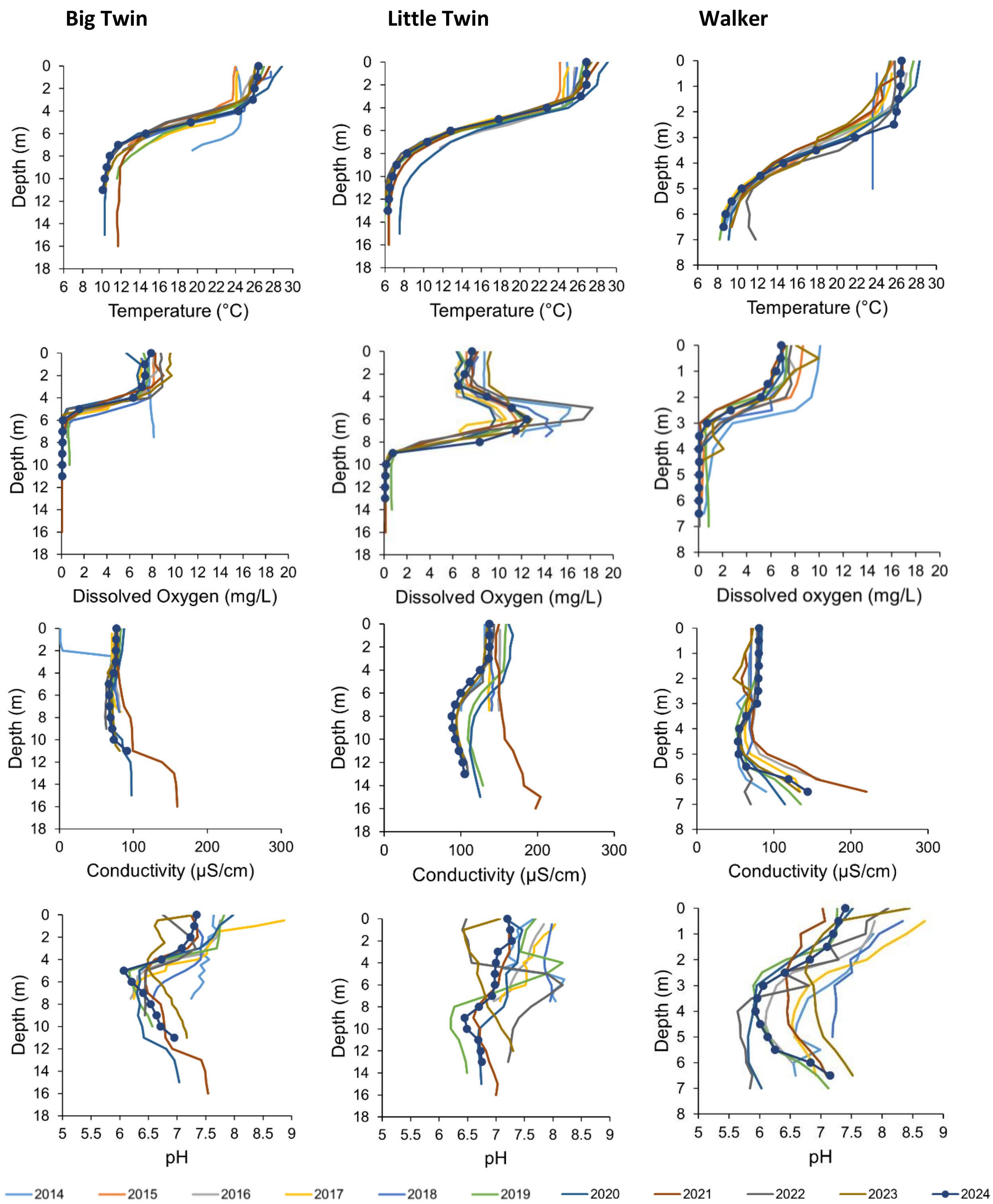
F. Zooplankton and phytoplankton community analysis

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 2 m through the surface with two tows 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 2014-2024 in TWCWC lakes. Note differences in Y axes among lakes. Dark blue lines with markers indicate the most recent year: 2024.

Appendix III: Literature Cited

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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.