



To the Twin and Walker Creeks Watershed Conservancy

Report of 2025 PLEON Sampling

From the Pocono Lakes Ecological Observatory Network

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I. Summary 2025: Twin and Walker Lakes at a Glance

PLEON partnered with TWCWC to monitor Big Twin, Little Twin, and Walker lakes five times in 2025 with PLEON on-site in July.

Table 1: Summary of 2025 monitoring.

	Variables Monitored	Crew
17 May 18 May	<ul style="list-style-type: none"> • Profiles: temperature, dissolved oxygen, conductivity, pH • Secchi Depth • Chlorophyll a (0.5 m, metalimnion) • Total N, Total P (0.5 m, hypolimnion) • Dissolved nutrients (hypolimnion) 	Collection: TWCWC Analysis: PLEON
20 June 22 June	<ul style="list-style-type: none"> • Profiles: temperature, dissolved oxygen, conductivity, pH • Secchi Depth • Chlorophyll a (0.5 m, metalimnion) • Total N, Total P (0.5 m, hypolimnion) • Dissolved nutrients (hypolimnion) 	Collection: TWCWC Analysis: PLEON
21 July	<ul style="list-style-type: none"> • Profiles: temperature, dissolved oxygen, conductivity, pH, light • Secchi Depth • Chlorophyll a (0.5 m, metalimnion) • Total N, Total P (0.5 m, hypolimnion) • Dissolved nutrients (hypolimnion) • Zooplankton and phytoplankton community (Twin Lakes only) 	Theresa Black (PLEON Manager) Ian Mahalek (PLEON Intern) Marlee Olsson (PLEON Intern)
15 Aug 17 Aug	<ul style="list-style-type: none"> • Profiles: temperature, dissolved oxygen, conductivity, pH • Secchi Depth • Chlorophyll a (0.5 m, metalimnion) • Total N, Total P (0.5 m, hypolimnion) • Dissolved nutrients (hypolimnion) 	Collection: TWCWC Analysis: PLEON
19 Sept 20 Sept	<ul style="list-style-type: none"> • Profiles: temperature, dissolved oxygen, conductivity, pH • Secchi Depth • Chlorophyll a (0.5 m, metalimnion) • Total N, Total P (0.5 m, hypolimnion) • Dissolved nutrients (hypolimnion) 	Collection: TWCWC Analysis: PLEON

Table 2: Summary of Big Twin Lake in 2025

	17 May	20 June	21 July	15 Aug	19 Sept
Thermally stratified?	YES	YES	YES	YES	YES
Epilimnion depth (m)	2	4	3	3	5
Metalimnion depth (m)	4	6	8	8	9
Secchi depth (m)	2.0	1.7	2.5	2.45	2.0
Vertical extinction coefficient (k)	—	—	0.84	—	—
Z_{10%} (m)	—	—	2.73	—	—
Z_{1%} (m)	—	—	5.47	—	—
Mean hypolimnetic DO (mg/L)	4.70	1.19	0.06	0.26	0.38
Epilimnetic chlorophyll (µg/L)	3.91	6.58	3.25	5.02	5.61
Epilimnetic TN (mg/L)	0.12	0.31	0.28	0.27	0.28
Epilimnetic TP (µg/L)	5.40	5.65	10.98	7.35	10.60
TSI_{secchi}	50.0	52.4	46.8	47.1	50.0
TSI_{chlorophyll}	44.0	49.1	42.2	46.4	47.5
TSI_{TP}	28.5	29.1	38.7	32.9	38.2
Trophic classification*	MESO**	MESO	MESO	MESO	MESO

*according to TSI_{chlorophyll}

**MESO = mesotrophic

Table 3: Summary of Little Twin Lake in 2025

	17 May	20 June	21 July	15 Aug	19 Sept
Thermally stratified?	YES	YES	YES	YES	YES
Epilimnion depth (m)	1	2	3	3	4
Metalimnion depth (m)	8	9	9	9	10
Secchi depth (m)	2.7	2.1	3.5	3.4	3.4
Vertical extinction coefficient (k)	—	—	0.58	—	—
Z_{10%} (m)	—	—	4.00	—	—
Z_{1%} (m)	—	—	8.00	—	—
Mean hypolimnetic DO (mg/L)	4.02	1.63	0.05	0.69	0.46
Epilimnetic chlorophyll (µg/L)	1.08	1.67	1.42	1.33	2.24
Epilimnetic TN (mg/L)	0.08	0.27	0.23	0.24	0.24
Epilimnetic TP (µg/L)	BD	BD	6.09	BD	5.04
TSI_{secchi}	45.7	49.3	41.9	42.4	42.4
TSI_{chlorophyll}	31.3	35.6	34.1	33.4	38.5
TSI_{TP}	—	—	30.2	—	27.5
Trophic classification*	OLIGO**	OLIGO	OLIGO	OLIGO	OLIGO

*according to TSI_{chlorophyll}

**OLIGO = oligotrophic

Table 4: Summary of Walker Lake in 2025

	18 May	22 June	21 July	17 Aug	20 Sept
Thermally stratified?	YES	YES	YES	YES	NO
Epilimnion depth (m)	1.5	1	2	1.5	NA
Metalimnion depth (m)	5.5	5.5	5.5	5.5	NA
Secchi depth (m)	1.2	1.1	1.25	2.0	1.7
Vertical extinction coefficient (k)	—	—	1.63	—	—
Z_{10%} (m)	—	—	1.41	—	—
Z_{1%} (m)	—	—	2.82	—	—
Mean hypolimnetic DO (mg/L)	0.62	0.59	0.02	0.29	NA
Epilimnetic chlorophyll (µg/L)	8.93	20.43	14.32	8.68	5.96
Epilimnetic TN (mg/L)	0.145	0.351	0.344	0.314	0.330
Epilimnetic TP (µg/L)	15.18	14.81	23.68	22.95	19.98
TSI_{secchi}	57.4	58.6	56.8	50.0	52.4
TSI_{chlorophyll}	52.1	60.2	56.7	51.8	48.1
TSI_{TP}	43.4	43.0	49.8	49.3	47.3
Trophic classification*	EUTRO**	EUTRO	EUTRO	EUTRO	MESO***

*according to TSI_{chlorophyll} **EUTRO = eutrophic ***MESO = mesotrophic

II. Chemical Profiles

A. Temperature

Big Twin was thermally stratified during all 2025 samplings (Figure 1). The epilimnion, or the well-mixed surface layer, extended to 2 m, 4 m, 3 m, 3 m, and 5 m during the May, June, July, August, and September samplings, respectively. The average epilimnetic temperature (\pm standard deviation) in Big Twin was 17.7°C (± 0.20) during the May sampling, 20.3°C (± 0.12) during the June sampling, 25.5°C (± 0.10) during the July sampling, 26.1°C (± 0.46) during the August sampling, and 20.6°C (± 0.45) during the September sampling. The metalimnion, or middle layer of rapid temperature change, extended to 4 m, 6 m, 8 m, 8 m, and 9 m during the May, June, July, August and September samplings, respectively.

Little Twin was thermally stratified during all 2025 samplings (Figure 1). The epilimnion extended to 1 m, 2 m, 3 m, 3 m, and 4 m during the May, June, July, August and September samplings, respectively. The average epilimnetic temperature (\pm standard deviation) in Little Twin was 20.0°C (± 0.0) during the May sampling, 21.4°C (± 0.17) during the June sampling, 26.2°C (± 0.30) during the July sampling, 26.3°C (± 0.39) during the August sampling, and 21.0°C (± 0.15) during the September sampling. The metalimnion extended to 8 m, 9 m, 9 m, 9 m, and 10 m during the May, June, July, August and September samplings, respectively.

Walker was thermally stratified during the May, June, July, and August 2025 samplings (Figure 1). The epilimnion extended to 1.5 m, 1 m, 2 m, and 1.5 m during the May, June, July, and August samplings, respectively. The average epilimnetic temperature (\pm standard deviation) in Walker was 17.9°C (± 0.12) during the May sampling, 22.0°C (± 0.49) during the June sampling, 25.5°C (± 0.11) during the July sampling, and 26.2°C

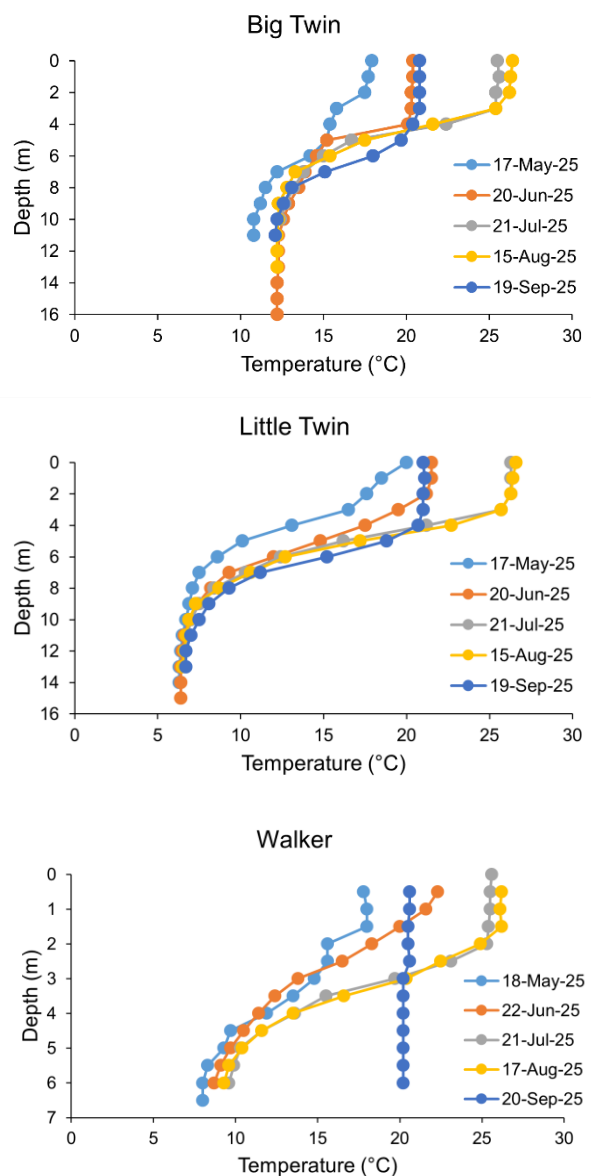


Figure 1: Temperature depth profiles for TWCWC lakes during 2025. Note scale difference among Y-axes.

(±0.06) during the August sampling. The metalimnion extended to 5.5 m during the May, June, July, and August samplings. Walker was not thermally stratified in September. September water temperatures ranged from 20.6°C at the surface to 20.2°C at 6 m depth.

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 as seen in the September sampling at Walker Lake.

B. Dissolved Oxygen

Big Twin was oxygenated through the epilimnion during all 2025 samplings (Figure 2). Dissolved oxygen concentration (DO) declined through the metalimnion during all samplings. Average DO concentration in the hypolimnion, or deep water, was 4.70 mg/L during the May sampling, 1.19 mg/L during the June sampling, 0.06 mg/L during the July sampling, 0.26 mg/L during the August sampling, and 0.38 mg/L during the September sampling. The depth at which DO concentration was below 2 mg/L, the threshold for oxygen depletion (called hypoxia), was 11 m, 9 m, 5 m, 5 m, and 6 m during the May, June, July, August, and September samplings, respectively.

Little Twin was also oxygenated through the epilimnion during all 2025 samplings (Figure 2). The maximum DO occurred in the metalimnion in May, June, July, and August with maximum concentrations at 5 m in May and 7 m in June, July, and August. DO concentration declined at depths below these maxima. September DO was greatest at the surface, declined, then increased at 6 m followed by a decline in DO to the sediments. Average DO in the hypolimnion was 4.02 mg/L during the May sampling, 1.63 mg/L during the June sampling, 0.05 mg/L during the July sampling, 0.69 mg/L during the August sampling, and 0.46 mg/L during the September sampling. DO concentration was below 2 mg/L, the threshold for

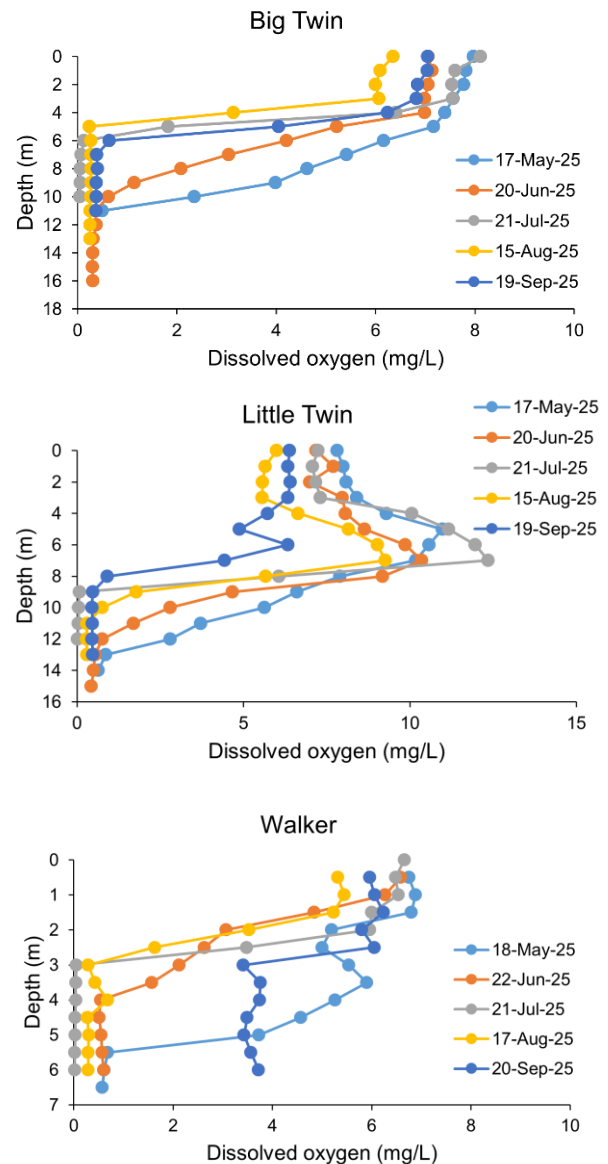


Figure 2: Dissolved oxygen profiles for TWCWC lakes during 2025. Note scale differences among the Y-axes.

oxygen depletion (called hypoxia), was 13 m, 11 m, 9 m, 9 m, and 8 m during the May, June, July, August, and September samplings, respectively.

Walker Lake was oxygenated through the epilimnion and DO generally declined through the metalimnion during all 2025 samplings (Figure 2). Average hypolimnetic DO concentration was 0.62 mg/L during the May sampling, 0.59 mg/L during the June sampling, 0.11 mg/L during the July sampling, and 0.06 mg/L during the August sampling. Walker was not stratified in September. DO at the sediments was 3.72 mg/L and did not fall below the 2.0 mg/L threshold for oxygen depletion.

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 66.9-85.8 $\mu\text{S}/\text{cm}$ during the May sampling, 66.7-96.4 $\mu\text{S}/\text{cm}$ during the June sampling, from 65.8-77.7 $\mu\text{S}/\text{cm}$ during the July sampling, from 70.2-100.6 $\mu\text{S}/\text{cm}$ during the August sampling, and 70.6-120.8 $\mu\text{S}/\text{cm}$ during the September 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 $\mu\text{S}/\text{cm}$ (Figure 3). Conductivity increased in the deep waters during May, June, August, and September. In July, conductivity decreased through the metalimnion, then increased in the hypolimnion. Conductivity ranged from 113.7-128.1 $\mu\text{S}/\text{cm}$ during the May sampling, 113.7-145.6 $\mu\text{S}/\text{cm}$ during the June sampling, from 90.9-131.4 $\mu\text{S}/\text{cm}$ during the July sampling, from 117.9-164.4 $\mu\text{S}/\text{cm}$ during the August sampling, and from 118.6-159.4 $\mu\text{S}/\text{cm}$ during the September sampling.

Conductivity of Walker was generally stable in the epilimnion. In May, June, and August, conductivity increased through the metalimnion and down to the sediments (Figure 3). In July, conductivity decreased in the metalimnion, then increased in the deep waters. September conductivity was consistent down the water column. Conductivity ranged from 59.9-108.4 $\mu\text{S}/\text{cm}$ during the May sampling, from 60.1-131.1 $\mu\text{S}/\text{cm}$ during the June sampling, from 65.0-111.9 $\mu\text{S}/\text{cm}$ during the July sampling, from 77.7-190.7

$\mu\text{S}/\text{cm}$ during the August sampling, and from 79.1-82.5 $\mu\text{S}/\text{cm}$ during the September 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 2025 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 6.12-7.47 during the May sampling, from 5.70-7.33 during the June sampling, from 6.12-6.97 during the July sampling, from 5.98-8.07 during the August sampling, and from 6.24-7.71 during the September 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 6.17-8.04 during the May sampling, from 6.11-8.11 during the June sampling, from 6.38-7.10 during the July sampling, from 6.36-7.59 during the August sampling, and 6.53-8.13 during the September sampling (Figure 4). Little Twin pH was more stable than Big Twin and Walker. pH decreased slowly through the epilimnion and metalimnion during the May, June, August, and September samplings. pH then increased slightly in the hypolimnion. During the July sampling, pH was lower in the epilimnion relative to other sampling dates and was steady throughout the epilimnion. The metalimnion and hypolimnion in July followed a pattern similar to other 2025 sampling dates.

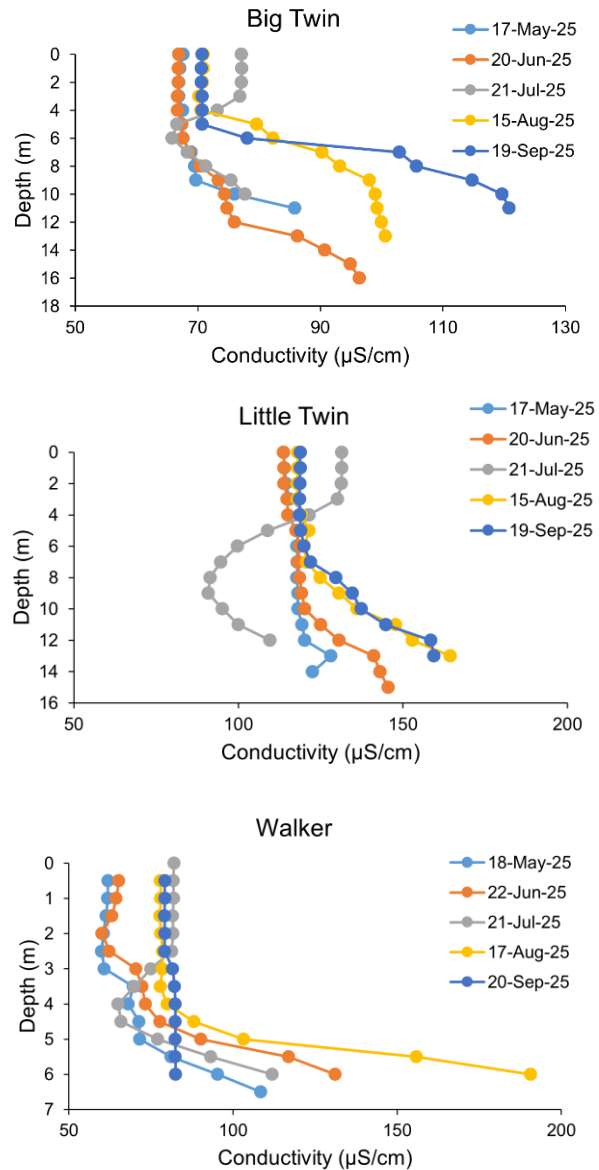


Figure 3: Conductivity depth profiles for TWCWC lakes during 2025. Note scale differences among the Y-axes.

pH in Walker ranged from 5.79-6.47 during the May sampling, from 5.53-7.33 during the June sampling, from 5.88-6.76 during the July sampling, from 5.73-7.41 during the August sampling, and from 6.50-7.89 during the September sampling (Figure 4). pH in Walker, like Big Twin, was lowest in the metalimnion between 3 m and 4 m depth during the May, June, July, and August sampling dates. pH decreased through the epilimnion, decreased more rapidly through the metalimnion, and increased through the hypolimnion. During the September sampling, pH decreased down the water column, with the lowest pH near the sediments, at 6 m depth.

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 6-9¹. 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 lakes². This was seen in some TWCWC profiles.

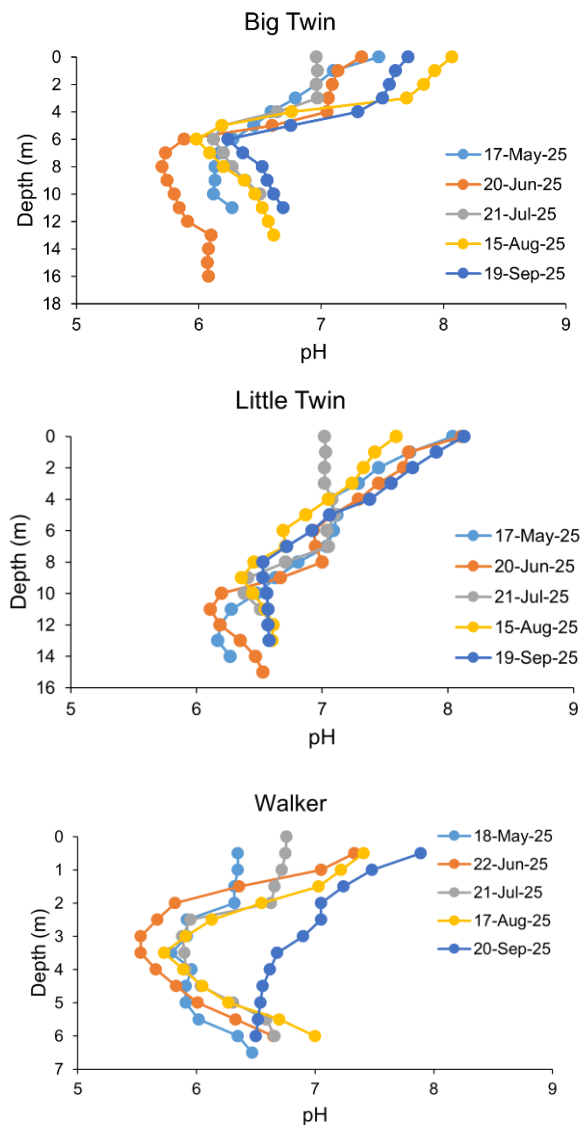


Figure 4: pH depth profiles for TWCWC lakes during 2025. Note scale differences among Y-axes.

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 equation³:

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

Secchi depth in Big Twin was 2.0 m, 1.7 m, 2.5 m, 2.45 m, and 2.0 m during the May, June, July, August, and September samplings, respectively (Figure 5). TSI_{Secchi} of Big Twin across these samplings was 50.0, 52.4, 46.8, 47.1, and 50.0, respectively, classifying Big Twin as eutrophic for May, June, and September and mesotrophic for the July and August samplings (Table 5).

Secchi depth in Little Twin was 2.7 m, 2.1 m, 3.5 m, 3.4 m, and 3.4 m during the May, June, July, August, and September samplings, respectively (Figure 5). TSI_{Secchi} of Little Twin across these samplings was 45.7, 49.3, 41.9, 42.4, and 42.4, respectively, classifying Little Twin as mesotrophic during all sampling dates (Table 5).

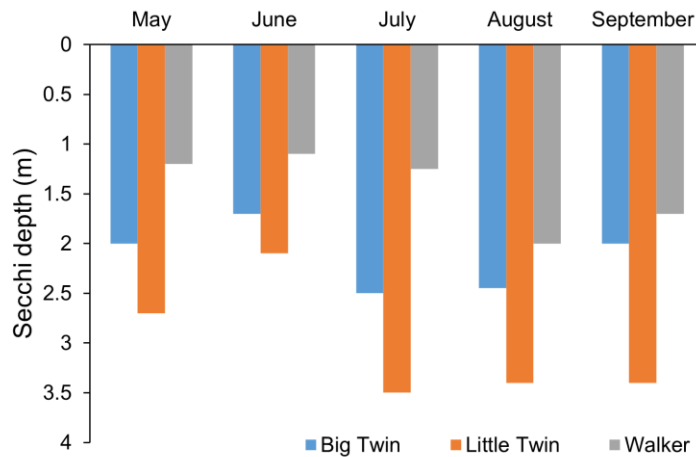


Figure 5: Secchi Depth for TWCWC lakes during summer of 2025. Sampling dates are summarized in Table 1.

Walker was generally the least clear TWCWC lake on all 2025 sampling dates (Figure 5).

Secchi depth in Walker was 1.2 m, 1.1 m, 1.25 m, 2.0 m, and 1.7 m during the May, June, July, August, and September samplings, respectively. TSI_{Secchi} of Walker across these samplings was 57.4, 58.6, 56.8, 50.0, and 52.4, respectively, classifying Walker as eutrophic on all sampling dates (Table 5).

Table 3: 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 2025 sampling. Little Twin was the clearest of the TWCWC lakes at this time ($k = 0.58$, $Z_{10\%} = 4.00$, $Z_{1\%} = 8.00$), followed by Big Twin ($k = 0.84$, $Z_{10\%} = 2.73$, $Z_{1\%} = 5.47$), and Walker ($k = 1.63$, $Z_{10\%} = 1.41$, $Z_{1\%} = 2.82$).

IV. Chlorophyll Results

Chlorophyll *a* (chl_a) is a pigment found in algal cells and is used as a proxy for algal abundance and lake productivity. PLEON measured chl_a concentration in the surface (0.5 m) and within the metalimnion of each lake.

Chl_a concentration at 0.5 m in Big Twin ranged from 3.25 µg/L to 6.58 µg/L over the 2025 samplings (Figure 6). The greatest epilimnetic chl_a concentration occurred in June and the lowest concentration was in July. Metalimnion samples in Big Twin were taken at depths between 4 and 7 m. Chlorophyll concentration in the metalimnion samples were lower than epilimnetic samples in May and June. Metalimnetic chl_a concentration was higher in the metalimnion in July, August, and September. Concentrations ranged from 1.54 µg/L in June to 12.72 µg/L in July.

Chl_a concentration at 0.5 m in Little Twin ranged from 1.08 µg/L to 2.24 µg/L over the 2025 samplings (Figure 6). Algal abundance in the epilimnion was greatest during the September sampling and lowest during the May sampling. Metalimnion samples were taken from depths between 6 m and 8 m. Chl_a concentrations were greater in the metalimnion for all months sampled, ranging from 4.99 µg/L (in August) to 23.15 µg/L (in May). This corresponds with the increased DO in the metalimnion.

Walker was the most productive of the TWCWC lakes during the summer of 2025; note the larger scale for the Y-axis for chl_a concentration (Figure 6). Chl_a concentration at 0.5 m in Walker ranged from 5.96 µg/L to 20.43 µg/L, with the lowest concentration in September and the greatest concentration in June. Metalimnetic samples were taken from depths between 2.5 m and 4.0 m. These samples had concentrations lower than those found in the epilimnion on the May, June, and July sampling dates. Metalimnion samples had greater concentrations than epilimnion samples in August and September.

Concentrations in the mid-depths ranged from 2.21 µg/L in May to 23.82 µg/L in September.

TSI can be calculated from chlorophyll *a* concentrations measured at 0.5 m according to the following equation³:

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

The $TSI_{chlorophyll}$ of Big Twin was 44.0, 49.1, 42.2, 46.4, and 47.5 during the May, June, July, August, and September samplings, respectively, classifying Big Twin as mesotrophic (Table 5).

The $TSI_{chlorophyll}$ of Little Twin was 31.3, 35.6, 34.1, 33.4, and 38.5 during the May, June, July, August, and September samplings, respectively, classifying Little Twin as oligotrophic (Table 5).

The $TSI_{chlorophyll}$ of Walker was 52.1, 60.2, 56.7, 51.8, and 48.1 during the May, June, July, August, and September samplings, respectively. These TSI values classify Walker as eutrophic in May, June, July, and August and as mesotrophic in September (Table 5).

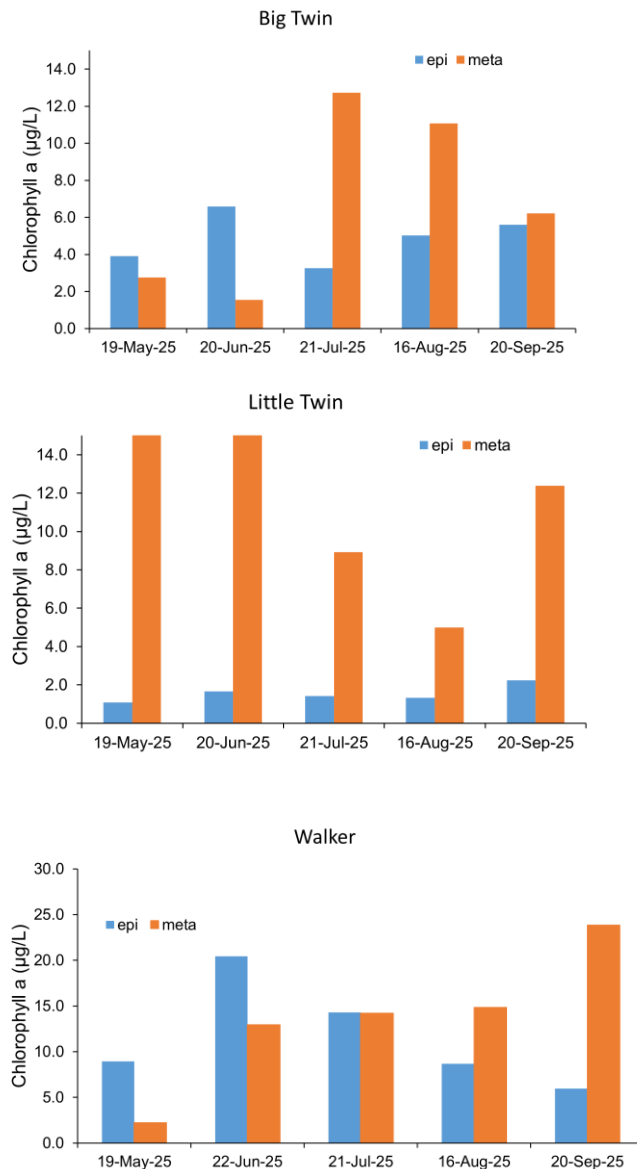


Figure 6: Chlorophyll-*a* concentrations in TWCWC lakes during 2025. Note scale differences among Y-axes and sampling dates among lakes (summarized in Table 1).

V. Nutrient Results

A. Total nitrogen

Total nitrogen concentration (TN) in samples collected from 0.5 m in Big Twin ranged from 0.12 mg/L to 0.31 mg/L, peaking in June (Figure 7). TN was greater in surface samples than deep water samples in May, June, and July. Deep water samples had a greater TN concentration than surface samples in August and September.

TN in samples collected from 0.5 m in Little Twin ranged from 0.08 mg/L to 0.27 mg/L during 2025 (Figure 7). Hypolimnion samples had greater TN compared to surface samples in Little Twin on all sampling dates after May. TN in hypolimnetic samples was 2x the epilimnetic concentration in September.

TN in samples collected from 0.5 m in Walker ranged from 0.15 mg/L to 0.35 mg/L during the summer of 2025 (Figure 7). Hypolimnion samples had greater TN compared to surface samples in Walker on all sampling dates after May. TN in hypolimnetic samples was 3x the epilimnetic concentration in June and 2x the epilimnetic concentration in July.

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 pollution¹.

B. Total phosphorus

Total phosphorus concentration (TP) in samples collected from 0.5 m in Big Twin ranged from 5.40 µg/L to 10.98 µg/L during the 2025 samplings (Figure 8). The greatest epilimnetic TP concentration occurred during the July sampling. TP concentrations in the hypolimnion were greater than those of the 0.5 m samples suggesting TP was more

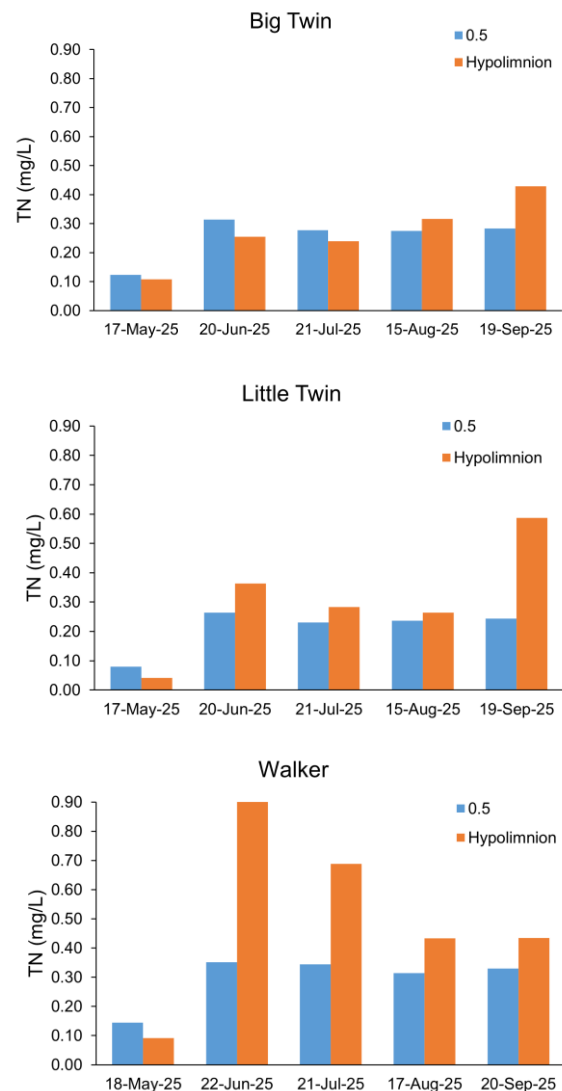


Figure 7: TN concentrations of water samples collected at varying depths for TWCWC lakes for the summer of 2025.

concentrated at deeper depths. The highest hypolimnetic TP concentration occurred in September, at 43.40 µg/L.

TP in samples collected from 0.5 m in Little Twin ranged from below detection to 6.09 µg/L across all 2025 samplings (Figure 8). Hypolimnetic TP concentrations were higher than all surface samples. TP in the hypolimnion was greatest in September at 61.35 µg/L; 12x greater than the September surface TP concentration.

TP in samples from 0.5 m in Walker ranged from 14.8 µg/L to 23.7 µg/L during the 2025 samplings. (Figure 8). TP in the hypolimnion sample were greater than the 0.5 m samples in May, June and August, but slightly lower in July and September. TP in the hypolimnion was greatest in May at 81.6 µg/L; 5x greater than the May surface TP concentration.

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 Extension¹ during 2025 samplings. Hypolimnetic TP concentrations in the Twin lakes were closer to this threshold, exceeding it in May, June, and August in Big Twin; September in Little Twin; and May, June, and August in Walker.

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.

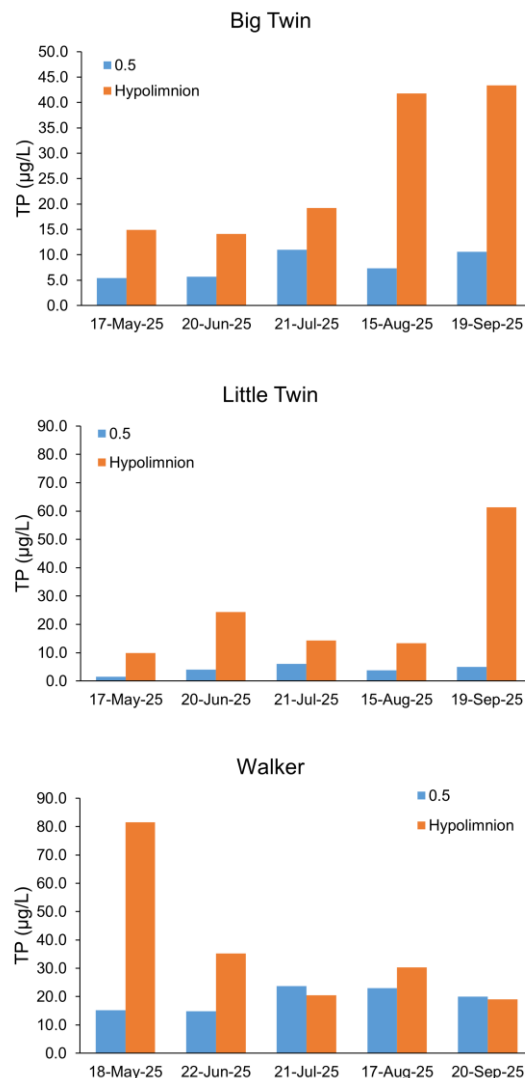


Figure 8: TP concentrations at varying depths for TWCWC lakes during the summer of 2025.

TSI can be calculated from TP concentration at 0.5 m as³:

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

TSI_{TP} of Big Twin was 28.5, 29.1, 38.7, 32.9, and 38.2 during the May, June, July, August, and September samplings, respectively. TSI_{TP} classified Big Twin as oligotrophic during all 2025 samplings (Table 5).

TSI_{TP} of Little Twin was 30.2 and 27.5 during the July and September samplings, respectively. TP was below detection in May, June, and August, so TSI_{TP} could not be calculated. TSI_{TP} classified Little Twin as oligotrophic during July and September (Table 5).

TSI_{TP} of Walker was 43.4, 43.0, 49.8, 49.3, and 47.3 during the May, June, July, August, and September samplings, respectively. TSI_{TP} classified Walker as mesotrophic during all 2025 samplings, though the July and August samplings were only slightly below the eutrophic range of the TSI (Table 5).

C. Dissolved nutrients

Dissolved inorganic forms of nitrogen and phosphorus are readily used by algae, bacteria, and aquatic plants. These forms are produced by a variety of physiochemical and biological processes, including runoff, decomposition, some forms of metabolism, and regeneration from sediments. The concentration of dissolved nutrients reflects the net of processes that generate and consume them. Dissolved nutrient concentrations were analyzed from hypolimnetic samples on each sampling date in 2025.

The dominant form of dissolved inorganic phosphorus in freshwater is typically orthophosphate (PO₄-P), or soluble reactive phosphorus (SRP). SRP concentration was below detection during all 2025 samplings (Table 6).

Nitrate (NO₃-N) and ammonium (NH₄-N) are common forms of dissolved nitrogen in lakes. Nitrate concentrations were above detection only in May 2025 in all three lakes.

Ammonium (NH₄-N) was detected in Big Twin and Little Twin on all 2025 sampling dates and in Walker on the May, June, and July 2025 sampling dates. (Table 6). Ammonium concentration in Big Twin ranged from 11.9 µg/L in June to 206.5 µg/L in September. Little Twin Concentrations ranged from 26.0 µg/L in July to 363.2 µg/L. Walker Lake concentrations of ammonium ranged from below detection in August and September to 677.6 in June.

It is typical for nitrogen to more commonly occur as ammonium (NH₄-N) in conditions of low oxygen. When oxygen is abundant, NH₄-N is typically transformed to NO₃-N. Both nitrogen forms are biologically available to algae; however, ammonium can become acutely toxic to fish at high levels, typically above 1,000 µg/L though this depends largely on the chemical and physical characteristics of the lake. Levels in the three lakes did not approach this concentration.

		$PO_4\text{-P}$ ($\mu\text{g/L}$)	$NH_4\text{-N}$ ($\mu\text{g/L}$)	$NO_3\text{-N}$ ($\mu\text{g/L}$)
Big Twin	17 May	BD	51.3	26.5
	20 June	BD	11.9	BD
	21 July	BD	33.1	BD
	15 Aug	BD	136.0	BD
	19 Sep	BD	206.5	BD
Little Twin	29 May	BD	59.1	11.9
	18 June	BD	103.6	BD
	15 July	BD	26.0	BD
	13 Aug	BD	134.0	BD
	18 Sep	BD	363.2	BD
Walker	29 May	BD	129.9	18.5
	30 June	BD	677.6	BD
	15 July	BD	441.8	BD
	13 Aug	BD	BD	BD
	18 Sep	BD	BD	BD

Table 6: Phosphate ($PO_4\text{-P}$), Ammonium ($NH_4\text{-N}$), and nitrate ($NO_3\text{-N}$) concentration in TWCWC Lakes during 2025 in hypolimnetic samples. Concentrations are averages of two replicates. BD = below detection, used when both reps were below detection limits.

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 21 July 2025. Walker was not sampled for zooplankton.

Zooplankton numbers in both lakes were dominated by rotifers, which made up 68% 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 (25% biomass in Big Twin, 15% biomass in Little Twin). Copepods made up 7% and 10% of zooplankton density 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 14.5 and 13.0, respectively and average diversity (Shannon-Wiener Index) was 0.86 and 0.77, respectively.

Table 4: Zooplankton community in the Twin Lakes on July 21, 2025 (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	73	68%	21	25%	76	82%	11	15%
<i>Anuraeopsis</i>	0		0		3		0	
<i>Asplanchna</i>	3		16		2		6	
<i>Conochilus</i>	18		1		34		1	
<i>Euchlanis</i>	2		0		0		0	
<i>Filinia</i>	0		0		0		0	
<i>Hexarthra</i>	0		0		0		0	
<i>Kellicottia</i>	1		0		0		0	
<i>Keratella</i>	30		3		28		2	
<i>Polyarthra</i>	18		2		9		1	
<i>Synchaeta</i>	0		0		0		0	
<i>Trichocerca</i>	1		0		2		0	
COPEPODA	8	7%	26	31%	9	10%	21	29%
Copepoda-Cyclopoida	5		17		3		6	
<i>Cyclops</i>	2		5		2		4	
<i>Mesocyclops</i>	3		12		1		2	
Copepoda-Calanoida	0		0		0		0	
Other Copepoda-Naupli	3		9		6		16	
CLADOCERA	26	25%	36	43%	36	43%	16	21%
<i>Bosmina</i>	21		21		4		3	
<i>Ceriodaphnia</i>	5		12		4		11	
<i>Daphnia dubia</i>	0		0		0		1	
<i>Daphnia pulex/pulicaria</i>	0		0		0		0	
<i>Diaphanosoma</i>	1		1		0		0	
<i>Holopedium</i>	0		3		0		0	
OTHER ZOOPLANKTON	0	0%	0	0%	0	0%	26	35%
TOTAL	107		83		93		74	

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 21 July 2025.

Cyanophyta (cyanobacteria) were the numerically dominant group in both Big Twin (81.4% phytoplankton density) and Little Twin Lake (99.8% phytoplankton density). Chrysophyta were the second-most dominant group in Big Twin, making up 7.7% of

phytoplankton density, while Bacillariophyta made up 5.9%. In Little Twin, 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 *Aphanizomenon*, *Woronichinia*, and *Dolichospermum* were identified (listed in order of greatest abundant to least abundant). The Little Twin community consisted of *Aphanizomenon*, and *Woronichinia*.

Average phytoplankton taxonomic richness in Big Twin was 18.5 and average diversity (measured using the Shannon-Wiener Index) was 0.64. Planktonic richness in Little Twin was 9.0 and diversity was 0.06.

Table 7: Phytoplankton community in the Twin Lakes on July 21, 2025 (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	103	5.9%	64	7.5%	23	0.1%	8	0.2%
Centric Diatoms	44	2.5%	19	2.2%	0	0.0%	0	0.0%
Araphid Pennate Diatoms	59	3.4%	46	5.3%	23	0.1%	8	0.2%
CHLOROPHYTA	19	1.1%	67	7.9%	0	0.0%	0	0.0%
Cocoid/Colonial Chlorophytes	7	0.4%	1	0.1%	0	0.0%	0	0.0%
Filamentous Chlorophytes	1	0.0%	1	0.1%	3	0.0%	1	0.0%
Desmids	12	0.7%	67	7.8%	0	0.0%	0	0.0%
CHRYSOPHYTA	134	7.7%	82	9.5%	161	0.5%	192	4.3%
Flagellated Classic Chrysophytes	128	7.3%	80	9.3%	161	0.5%	192	4.3%
Tribophytes/Eustigmatophytes	6	0.3%	2	0.2%	0	0.0%	0	0.0%
Raphidophytes	1	0.0%	1	0.1%	3	0.0%	1	0.0%
CRYPTOPHYTA	33	1.9%	112	13.0%	18	0.1%	69	1.5%
CYANOPHYTA	1415	81.4%	159	18.5%	32760	99.8%	4196	93.0%
Unicellular and Colonial Forms	265	15.2%	3	0.3%	520	1.6%	5	0.1%
Filamentous Nitrogen Fixers	1150	66.1%	156	18.2%	32240	98.2%	4191	92.8%
Filamentous Non-Nitrogen Fixers	0	0.0%	0	0.0%	0	0.0%	0	0.0%
EUGLENOPHYTA	21	1.2%	16	1.8%	8	0.0%	8	0.2%
PYRRHOPHYTA	16	0.9%	440	51.3%	5	0.0%	234	5.2%
TOTAL	1739		858		32815		4515	

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. Additionally in 2025, TWCWC provided an elements analysis that was conducted on all three lakes in 2018 and 2019.

PLEON monitored DOC concentration in all three lakes from 2020-2024. These data are not included in this summary but have been presented in previous PLEON reports.

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 2014-2025.

TWCWC lakes were generally stratified in the summer months (June, July, August) from 2014-2025. Surface temperature in TWCWC lakes, while variable, have generally increased over this period, though not with statistical significance (Figure 9).

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

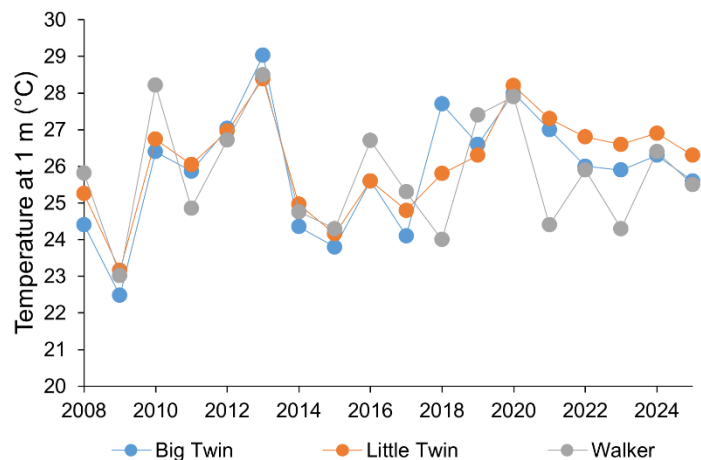


Figure 9: July temperature at 1 m in TWCWC lakes from 2008 to 2025.

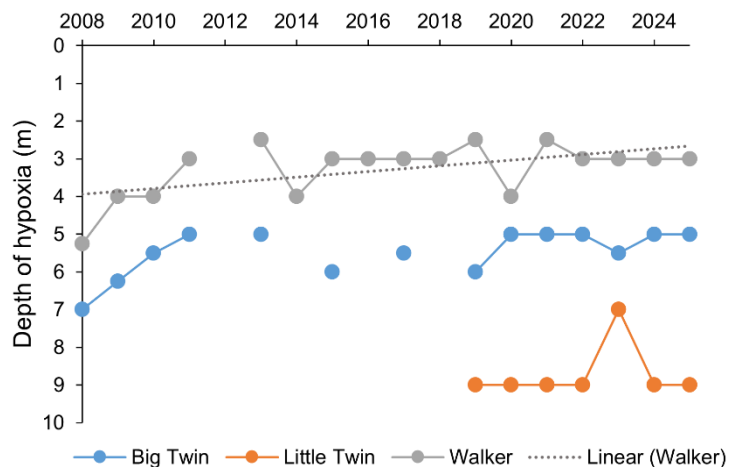


Figure 10: Depth of hypoxia in July for TWCWC lakes from 2008 to 2025. Missing data in Walker Lake indicate that there was no hypoxia while missing data for the Twin Lakes indicate incomplete profiles, not the absence of hypoxia.

2 mg/L (the threshold for oxygen depletion) was deepest in Little Twin and most shallow in Walker (Figure 10). The trendline for the Walker data indicates a significant decrease in the depth of DO depletion. 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

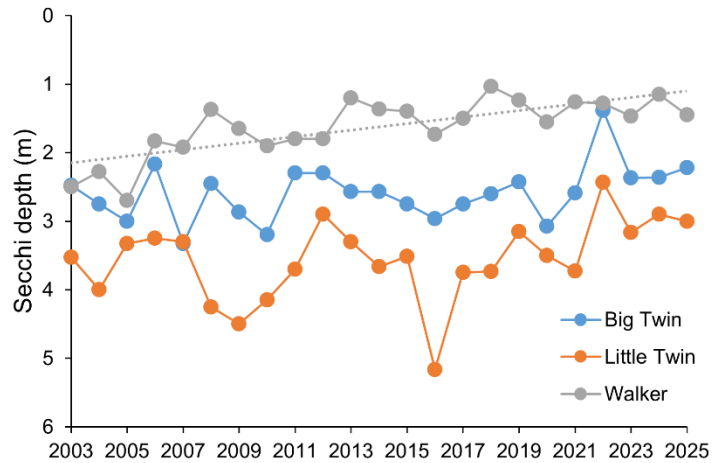


Figure 11: Average summer (June, July, August) Secchi depth for TWCWC lakes from 2003 to 2025.

consistently greater than that of Big Twin and Walker across the dataset, with an average conductivity of 142.3 $\mu\text{S}/\text{cm}$ compared to 82.3 $\mu\text{S}/\text{cm}$ and 78.9 $\mu\text{S}/\text{cm}$ in the other lakes, respectively (averages include all depths in June, July, and August of all years). These results are consistent with a 2018-19 analysis of salinity in the Twin Lakes (CITE REPORT). Salinity contributes to conductivity.

pH in Walker Lake was generally lower than that of Big Twin and Little Twin across the dataset, with an average pH of 6.66 compared to 6.99 and 7.18 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 2003-2025; Figure 11).

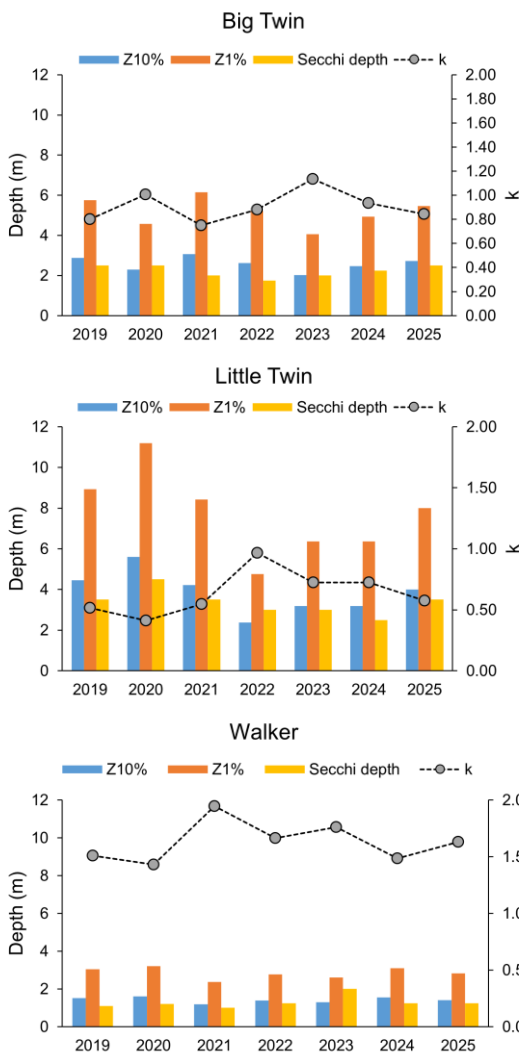


Figure 12: Light attenuation in TWCWC lakes from 2019-2025. Measurements made in July.

Secchi depth in Walker decreased over the 22-year dataset (linear regression, $r^2 = 0.57$, $p < 0.01$).

Light attenuation parameters have been measured in TWCWC lakes during the July samplings 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 $\mu\text{g/L}$ to 10.2 $\mu\text{g/L}$ in Big Twin, from 0.71 $\mu\text{g/L}$ to 8.2 $\mu\text{g/L}$ in Little Twin, and from 2.3 $\mu\text{g/L}$ to 19.3 $\mu\text{g/L}$ in Walker (Figure 13; top panel). Chla concentrations declined over time, with a statistically significant decline in Little Twin (linear regression, $r^2 = 0.63$, $p = <0.001$) and a general but not significant decline in Big Twin. Chla concentrations in Walker over time were much more variable.

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 0.22 mg/L to 0.49 mg/L in Big Twin, from 0.17 mg/L to 0.94 mg/L in Little Twin, and from 0.34 mg/L to 0.78 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 $\mu\text{g/L}$ to 32.0 $\mu\text{g/L}$ in Big Twin, from 3.5 $\mu\text{g/L}$ to 29.0 $\mu\text{g/L}$ in Little Twin, and from 11.2 $\mu\text{g/L}$ to 34.0 $\mu\text{g/L}$ in Walker (Figure 13; bottom panel).

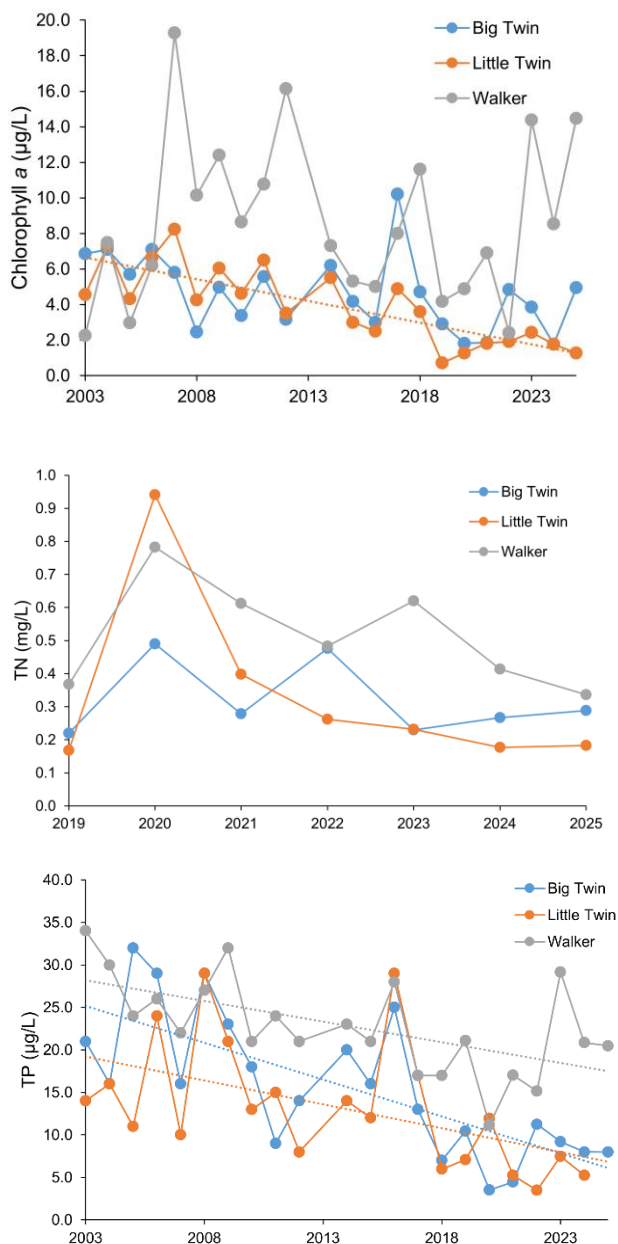


Figure 13: Average epilimnetic chla, TN, and TP in TWCWC lakes from 2003-2025. Dashed lines show significant trends over time.

Summer TP generally declined over the 22-year dataset in all lakes. This decline is statistically significant in Big Twin (linear regression, $r^2 = 0.53$, $p = <0.001$), Little Twin (linear regression, $r^2 = 0.32$, $p = 0.005$), and Walker (linear regression, $r^2 = 0.35$, $p = 0.004$). TP in Walker increased in 2023 and remained high relative to historical concentrations in 2024 and 2025.

F. Trophic status over time

Big Twin was generally mesotrophic since 2014 and Little Twin was generally oligo-mesotrophic (Figure 16). Walker Lake has been mesotrophic across most of the historical dataset; however, since 2023, the average summer $TSI_{chlorophyll}$ has been in the eutrophic range.

TSI_{Secchi} was typically greater than $TSI_{chlorophyll}$ and TSI_{TP} in all three lakes, particularly since 2018 when TSI_{TP} began declining. $TSI_{chlorophyll}$ also declined in Little Twin, but has been more variable in Big Twin and Walker.

Chlorophyll is direct measurement of algal abundance and is the most appropriate assessment of lake productivity, or trophic status. Many other factors, such as dissolved and suspended compounds, can affect Secchi depth. TSI_{Secchi} is greater than $TSI_{chlorophyll}$ in most PLEON lakes.

G. Zooplankton Over Time

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

Zooplankton density ranged from 107.5 to 540 individuals/L in Big Twin and from 52 to 341 individuals/L in Little Twin across the 7-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.



Figure 14: Average summer TSI in TWCWC lakes from 2003-2025 according to Secchi depth, chl_a, and TP. Horizontal lines show thresholds for oligotrophy (<40), mesotrophy (40-50), and eutrophy (>50).

Average zooplankton richness ranged from 10.5-14.5 in Big Twin and from 9.0-16.5 in Little Twin over the 7-year dataset (Figure 15). Zooplankton diversity ranged from 0.67-0.86 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.

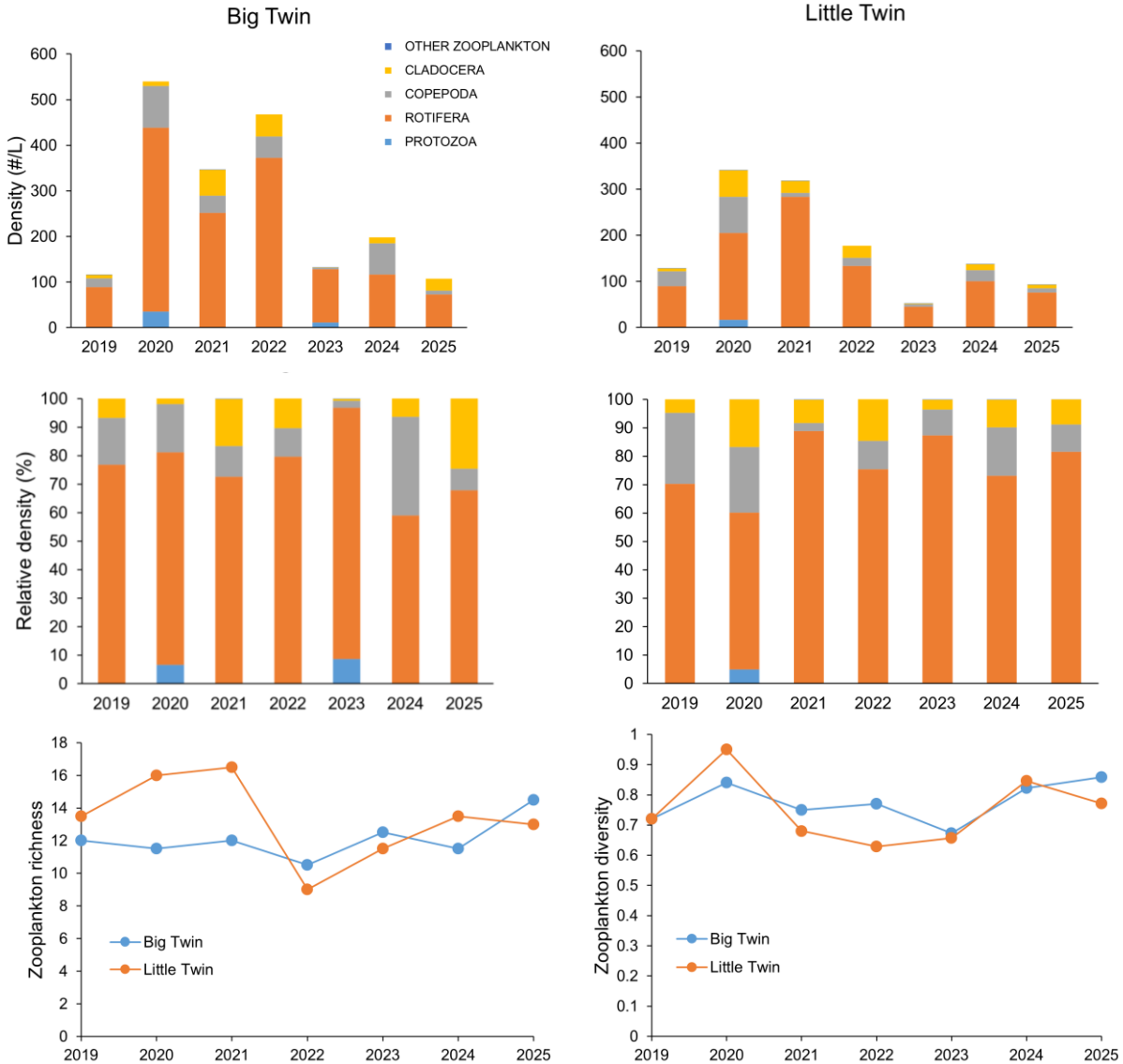


Figure 155: Zooplankton communities in the Twin Lakes from 2019-2025. Samples were collected in July of each year. Bars and symbols are averages of two replicates.

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 16). This increase was driven by an increase in the abundance of Cyanophyta, or cyanobacteria. Density in Big Twin has been variable since the 2022 high, with 2024 density the second greatest value seen across the 7-year data set. Density in Little Twin

decreased after 2022, then increased to the highest value in 2025. Cyanophyta make up a large percentage of phytoplankton density and were the dominant form in both lakes in 2025, making up 99.8% of phytoplankton density in Little Twin.

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. In 2024 and 2025, Cyanophyta was dominant, and diversity remained high. More years of sampling are needed to determine if this shift is due to variation or will become more “normal” for Big Twin Lake. Diversity in Little Twin decreased greatly in 2025 to a low value of 0.06 and was dominated by Cyanophyta.

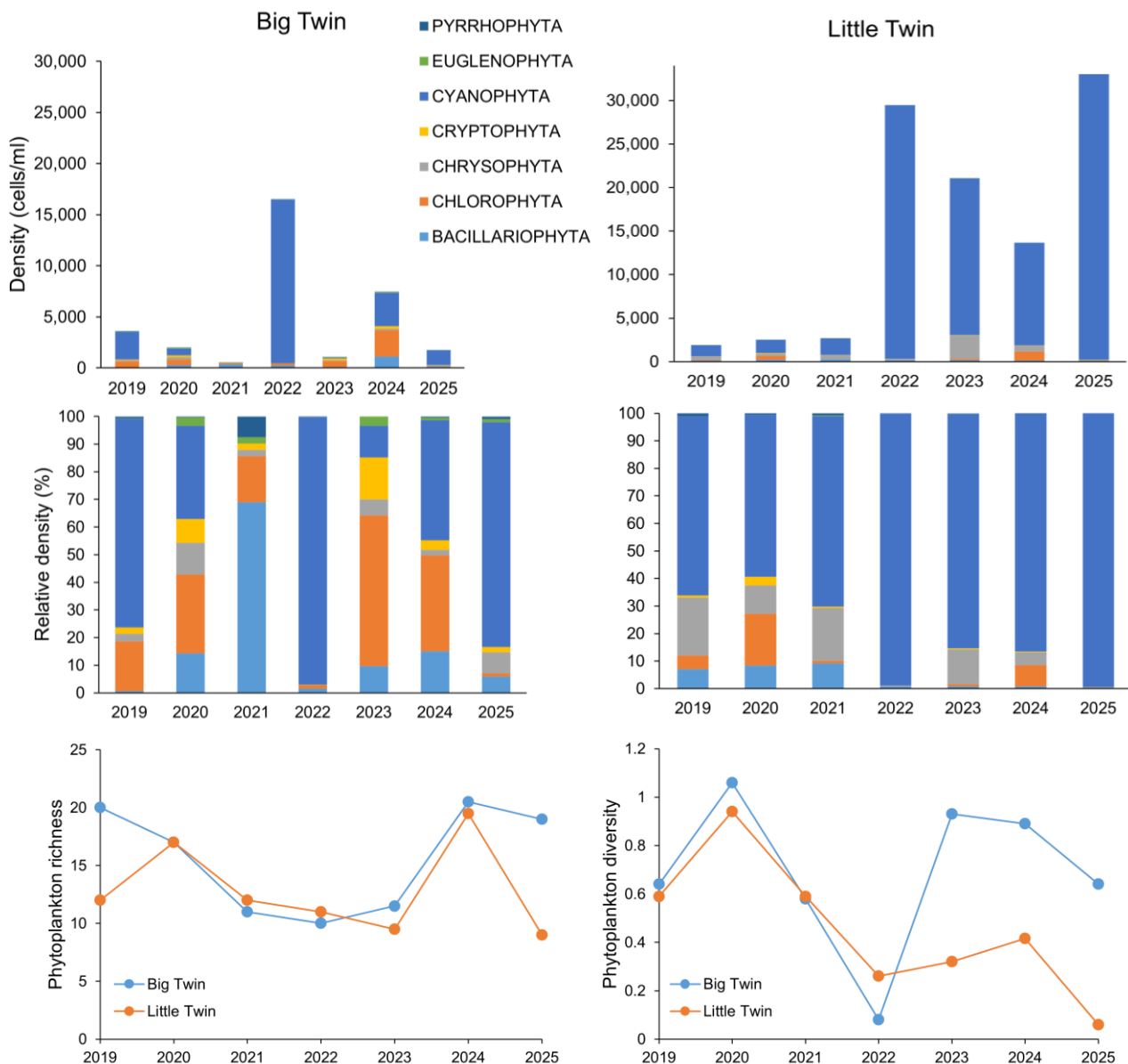


Figure 16: Phytoplankton communities in the Twin Lakes from 2019-2025. Samples were collected in July during each year. Bars and symbols are averages of two replicates.

I. 2025 Overview of Climate Conditions

A comprehensive understanding of lake ecology should include data collected over the range of temperature and precipitation typical to a lake's climate. This section provides a brief overview of climatic conditions from winter 2024-2025 through Fall 2025. This section is not site specific but covers the Pocono region, specifically highlighting Pike, Wayne, Monroe, Lackawanna, Susquehanna, and Schuylkill Counties. It also does not specify precipitation dates, times, or quantities but discusses monthly averages and deviations from the long-term average of available NOAA data⁴.

During the winter 2024-2025, the Poconos saw below average total precipitation (including snowfall) and below average temperatures of 2° to 4° F below the long-term average⁴. Though no formal data was gathered, several PLEON lakes reported full ice cover in January and February during this cold spell.

Spring began with a warm, dry March with temperatures 4° to 8° F above the monthly average. The first half of April saw below-average temperatures and above-average precipitation. In the second half of the month, precipitation decreased and temperatures warmed. May, however, saw some record-setting precipitation with much of eastern PA receiving over 200% of the typical monthly average. Temperatures were warm in the first half of the month, falling below average in the second half.

The month of June started cold but ended with a heat dome event, beginning on June 22nd that resulted in daytime temperatures nearing 100° F. Precipitation differed across the area with the western part of the Poconos including Wayne, Lackawanna, Susquehanna, Schuylkill and north west Monroe counties experiencing above average rainfall, while Pike and most of Monroe county experienced slightly below average rainfall.

July temperatures were 2° to 6° F above average, with greater anomalies in southern Wayne and northeastern Pike counties. The first half of July saw above average precipitation across the Poconos, ranging from 100 to 200% of normal amounts, and was particularly heavy in southeastern Pike and Monroe counties. The second half of July was dry across the area leading into an exceptionally dry August with the area seeing anywhere from 25 to 75% of normal rainfall. August was cooler than average across most of the Poconos, with deviations ranging from 0° to 2° F, mainly driven by a cold end to the month. Central Wayne County was the only section of the PLEON study area that experienced slightly above-average temperatures.

September was dry all around, with Pike, Monroe, and Schuylkill counties receiving less than 50% of normal rainfall. Most of Wayne, Lackawanna, and Susquehanna counties received from 50 to 75% of normal rainfall, with the Anthracite valley area and the very northern region of Wayne County receiving a bit more. September was 2° to 4° F above the average temperature across the region.

October was also warm and dry, with temperatures between 0° and 4° F above average and precipitation 50% to 100%, driven up by rainfall on the last few days of the month.

This dry spell continued in November with precipitation between 25% and 75% of the month’s long-term average. Temperatures across the Poconos varied in November but did not stray far from the historical monthly average, ranging from 2° F below to 2° F above the average.

As of early December 2025, Pike, Monroe, and Schuylkill Counties are under a drought watch, according to the PA Commonwealth Drought Task Force⁵. The US Drought Monitor has declared the Poconos as ranging from Abnormally Dry to Moderate Drought, with a small portion of southern Pike and southeastern Monroe Counties in Severe Drought⁶.

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

A. Description of PLEON Lakes

The PLEON dataset consists of 35 lakes in Pike, Wayne, Monroe, Lackawanna, Susquehanna, and Schuylkill Counties. Lakes range in surface area, shoreline, and depth (Figure 17). The 35 lakes sampled by PLEON have an average depth of 7.5 m; Big Twin and Little Twin are almost twice the average depth, while Walker is about a meter deeper than the PLEON average.

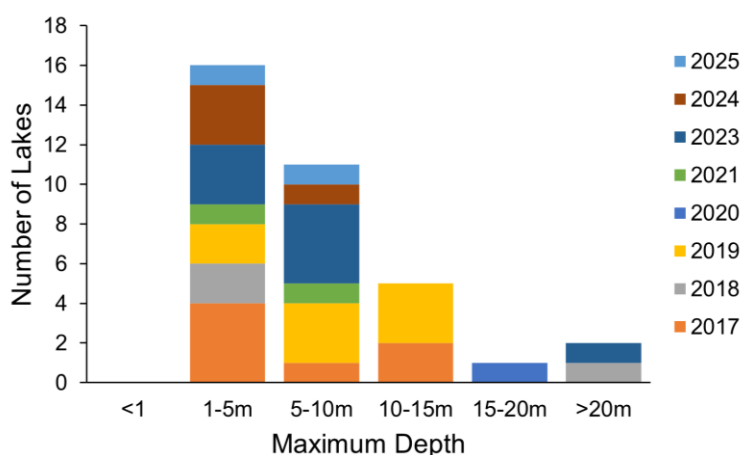


Figure 17: Maximum depth of PLEON lakes. Years refer to the first PLEON sampling year. Not all lakes are sampled every year.

B. Water transparency

PLEON recorded Secchi depth at least once during the summer months (June, July, August) of 2025 in 20 of the 35 lakes. The average summer Secchi depth in these lakes ranged from 0.75 m to 5.67 m with an average of 2.07 m (Figure 18). The average summer Secchi depth for Little Twin was deeper than the PLEON average, at 3.0 m. The average Secchi depth for Big Twin was close to the PLEON average, at 2.22 m. The Walker average summer Secchi depth was shallower than the PLEON average, at 1.45 m.

C. Lake productivity

Lake productivity, as measured by chl_a concentration at 0.5 m depth, was assessed in 20 PLEON lakes during the summer months (June, July, August) in 2025 (Figure 18). Average summer Chl_a concentration in these lakes ranged from 0.45 µg/L to 72.45 µg/L with an average of 11.24 µg/L and a median of 5.69 µg/L (Figure 19). Walker exceeded the average PLEON concentration by about 3 µg/L. Big Twin and Little Twin had summer average chl_a concentrations below the PLEON average and median; with Big Twin at about half the PLEON average concentration. Little Twin was among the least

productive of the PLEON lakes in 2025 and was about 1/8 the PLEON average and 1/4 of the PLEON median.

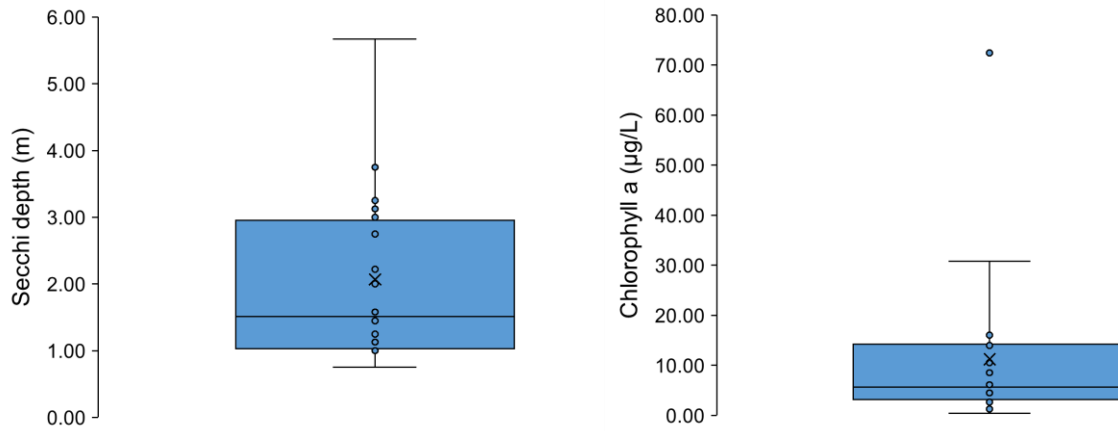


Figure 16: Average summer (June, July, August) Secchi depth (left) and chlorophyll a concentration at 0.5 m (right) across 20 PLEON lakes monitored in 2025. Lines within boxes are medians and X symbols are means. Upper and lower box boundaries denote the 75th and 25th percentile, respectively while upper 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 18 lakes during the summer months of June, July, and August 2025. Average summer TN concentration ranged from 0.171 mg/L to 1.07 mg/L in these lakes, with an average concentration of 0.400 mg/L and a median of 0.345 mg/L (Figure 19). The average summer TN for Big Twin (0.289 mg/L) and Little Twin (0.183 mg/L) and Walker (0.336 mg/L) were less than the PLEON average.

Total phosphorus (TP) concentration was quantified at 0.5 m depth in 20 PLEON lakes during the summer months (June, July, August) of 2025. Average summer TP concentration ranged from values below detection to 33.4 µg/L, with an average of 15.1 µg/L and a median of 12.2 µg/L (Figure 19). The summer average epilimnetic TP concentration in Big Twin and Little Twin were below the PLEON average, at 4.6 µg/L in Little Twin and 8.0 µg/L in Big Twin. The average summer epilimnetic TP concentration in Walker was above the PLEON average, at 20.5 µg/L.

E. Cyanobacteria

Since 2017, PLEON has collected 329 samples for PTOX screening as a part of its formal monitoring program. These samples were collected from 25 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.

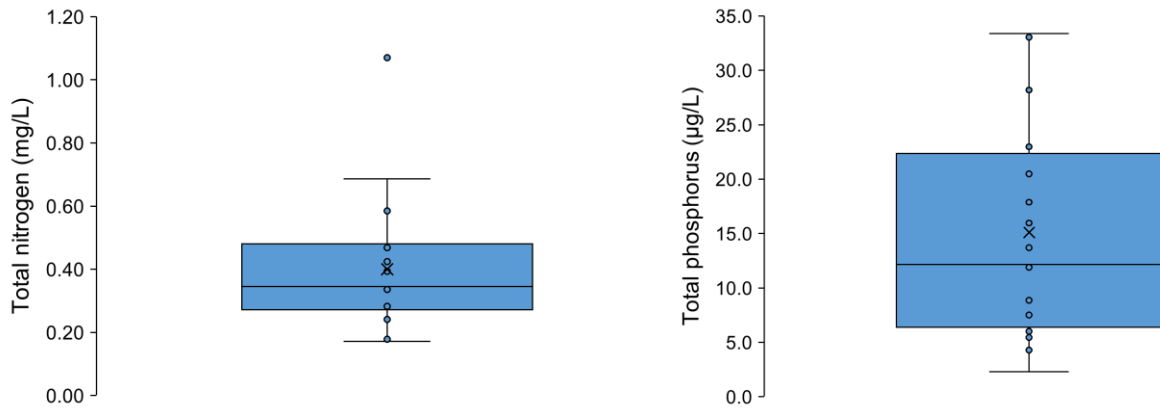


Figure 19: Average summer (J, J, A) TN and TP of PLEON lakes monitored in 2025. Lines are medians and X symbols are means. Upper and lower boundaries are the 75th and 25th percentile, respectively. Whiskers show maximum and minimum values. Circles are single measurements or an average if the lake was sampled more than once during the summer. Nutrient concentrations were quantified from 0.5 m depth.

Eleven (possibly 12, some specimens are difficult to identify) PTOX cyanobacteria genera have been found in PLEON samples to date (Figure 20). The most commonly found genera are *Dolichospermum*, followed by *Aphanizomenon* (or *Aphanizomenon*-like). *Chrysochloris*, *Woronichinia*, and *Microcystis* were also common. Ninety-six of the samples (or 29%) did not have PTOX taxa present. Four 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 *Chrysochloris* 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.

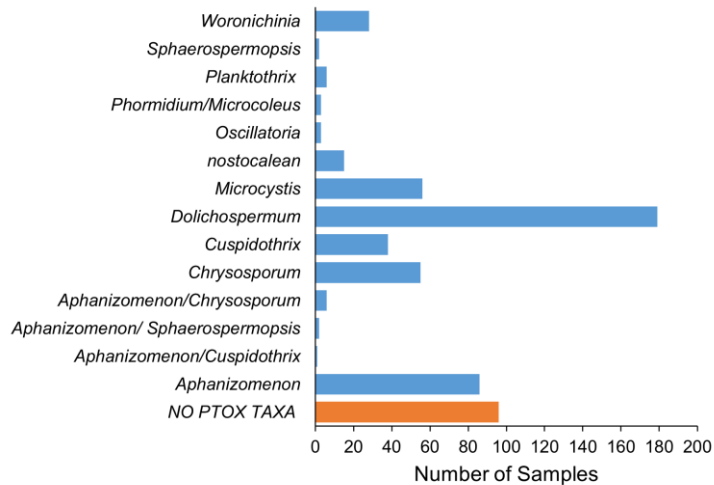


Figure 20: Potentially toxic (PTOX) cyanobacteria genera found in samples collected from PLEON lakes since 2017. PTOX screens were conducted by Greenwater Laboratories.

Based on the results of the PTOX screens, Greenwater Laboratories has recommended quantifying microcystin/nodularin concentration in 28% 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 65% and 75% of the recommended analyses have been conducted, depending on the toxin.

Microcystin/nodularins, cylindrospermopsin, anatoxin, 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 neurotoxin⁷. The US Environmental Protection Agency recommends microcystin and cylindrospermopsin magnitude thresholds of 8 µg/L (or ng/mL) and 15 µg/L in recreational waters⁸. The Lake Erie Harmful Algal Bloom Monitoring and Response Strategy recommends a Recreational Use Advisory when saxitoxin concentration is 0.8 µg/L or above⁹. 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. PTOX screens was not completed in 2025.

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	92	69	20	9.44	0.16-129
cylindrospermopsin	71	46	1	0.07	-
anatoxin-a	71	49	1	0.14	-
saxitoxin	71	50	6	0.45	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 2025 monitoring program should be highlighted:

1. 2025 data shows similar patterns in algal abundance and TP concentrations in all three lakes.

2025 was the first year in which chlorophyll and nutrient data were collected in May and September. Patterns in chlorophyll *a* concentration, a proxy for the amount of algae, differed in the three lakes. Big Twin *chl a* concentrations increased from May to August, then fell in September. Little Twin had a maximum metalimnetic *chl a* concentration in May, after which *chl a* declined through August and increased during the September sampling date. Walker *chl a* concentrations were more variable, with epilimnetic concentrations peaking in June, then falling through the summer and fall while metalimnetic *chl a* concentrations rose through the summer and peaked in September.

Availability of phosphorus has correlated with *chl a* concentration in the past in all three lakes. In 2025, total phosphorus concentrations followed similar patterns to *chl a* in each lake. TP concentration peaked in June in both Little Twin and Walker, after which concentrations decreased. Big Twin TP concentration increased from May to August, paralleling *chl a* concentrations.

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).

It is clear from the 2025 data that algal growth is starting well before summer and lasting into fall. Continued sampling in these months could determine long-term trends in seasonality and shed more light on a possible relationship to phosphorus availability.

2. Greater nutrient concentrations in the hypolimnion of all three lakes.

Nutrient regeneration is one of many sources that contribute to nutrient loading in lakes, particularly phosphorus. When the water overlying lake sediments is anoxic, phosphorus bound to other compounds is released and re-enters the water column and can fuel algal production. This process is common in deep lakes and can be problematic if it represents a major proportion of the lake's nutrient supply.

The hypolimnion of all three lakes were commonly anoxic during the summer months, which is one of the conditions needed for nutrient regeneration to occur. Big Twin, Little Twin, and Walker lakes had higher TP concentrations in hypolimnetic samples than epilimnetic samples throughout the summer. 2025 was the first year in which hypolimnetic samples were collected in Walker Lake. However, the overall contribution of internal phosphorus loading relative to other potential sources of phosphorus in these lakes is unknown.

3. 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, correlations remain weak to moderate (Figure 21). Average summer Secchi depth was not significantly correlated with average epilimnetic summer chlorophyll *a* concentration in any of the TWCWC lakes. There was a weak correlation coefficient of $r = 0.28$ in Little Twin, suggesting a weak positive relationship between *chl*_a and Secchi depth. Average epilimnetic chlorophyll *a* concentration correlated moderately ($r = 0.46$ in Little Twin) with epilimnetic TP concentration, but only weakly ($r = 0.29$) in Big Twin. *Chl*_a concentration did not respond strongly to TP concentration in Walker Lake (Figure 21).

There are several explanations for weaker correlations between these variables. 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 play 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.

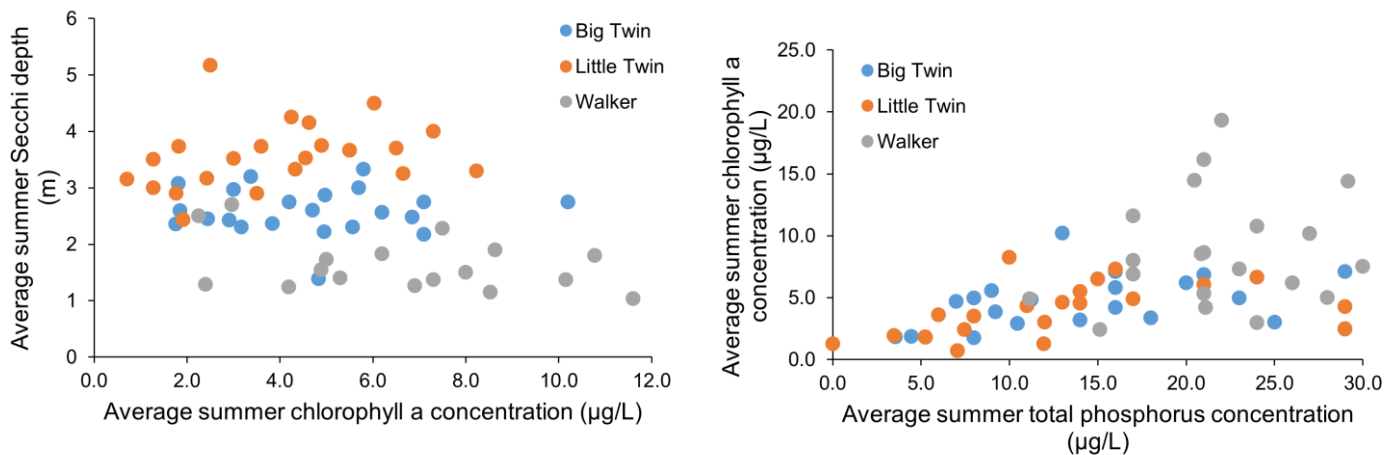


Figure 17: Correlations between average summer Secchi depth and average summer chlorophyll *a* and between average summer chlorophyll *a* and average summer TP in TWCWC lakes over the long-term dataset.

4. 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 ($r^2 = 0.61$). 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. PLEON has not collected data quantifying these compounds in Walker Lake, with the exception of the limited dissolved organic carbon data (collected from 2020 to 2024). DOC was 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. Other dissolved compounds, such as some metals, can influence water clarity, as can the amount of suspended sediments. Efforts to mitigate runoff and resuspension of sediments can reduce the amount of suspended sediments in lakes.

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

Potentially toxigenic cyanobacteria blooms remain a concern for all Pocono lakes and for the Twin and Walker Lakes specifically. There are several lines of evidence suggesting reasons for concern in TWCWC lakes:

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 *Chrysochloris*. 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](#).

Report of 2025 PLEON Sampling: Twin and Walker Lakes

APPENDICES

APPENDIX I: Description of Field Sampling Methods

A. Physical Profiles

Temperature, dissolved oxygen, conductivity, and pH were measured using a handheld YSI Professional Plus multiparameter instrument fitted with a polarographic dissolved oxygen probe and a pro series pH probe. Probes were calibrated monthly throughout 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 analyzed at Drexel University. 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-}).

Samples intended for dissolved nutrient analysis were filtered through ashed GF/F filters (Whatman, 0.7 μm pore size) and frozen at -20°C until analysis for nitrate (NO_3^- -N), ammonium (NH_4 -N), and phosphate (PO_4 -P).

NO₃-N concentration of the digested samples was quantified via cadmium reduction using a discrete autoanalyzer (AQ300, SEAL Analytical) at Drexel University (EPA method 353.2).

NH₄-N concentration of the digested samples was quantified via the indophenol blue colorimetric method using a discrete autoanalyzer (AQ300, SEAL Analytical) at Drexel University (EPA method 350.1)

PO₄-P concentration of the digested samples was quantified via the ascorbic acid colorimetric method using a discrete autoanalyzer (AQ300, SEAL Analytical) at Drexel University (EPA method 365.1).

D. 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.

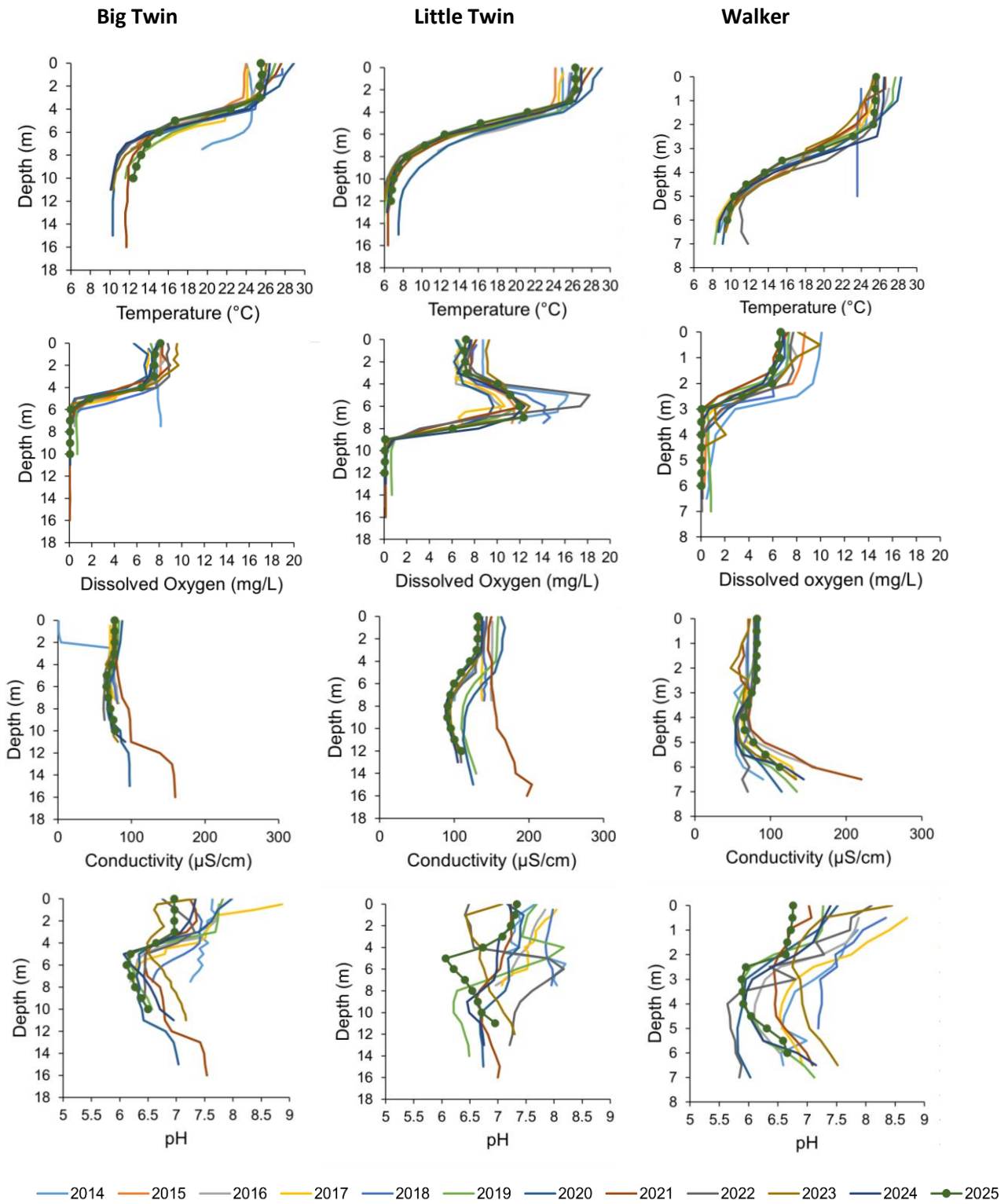
E. Zooplankton and phytoplankton community analysis

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 replicate samples were collected and 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 a depth 1 to 2 m from the sediments to 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 2014-2025 in TWCWC lakes. Note differences in Y axes among lakes. Dark green lines with markers indicate the most recent year: 2025.

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 $\mu\text{S}/\text{cm}$.

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.