To the Twin and Walker Creeks Watershed Conservancy

Report of 2019 PLEON Sampling

From the Pocono Lakes Ecological Observatory Network

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I. Summary 2019: Twin and Walker Lakes at a Glance

A. Description of monitoring activities

PLEON partnered with TWCWC to monitor Big Twin, Little Twin, and Walker lakes 4 times in 2019. TWCWC monitoring included profiles of temperature, DO, conductivity, pH, and TDS and samples for chlorophyll, TN, and TP (processed by PLEON).

Table 1. Summary of PLEON 2019 monitoring activities by lake.

	Big Twin	Little Twin	Walker		
TWCWC	22 June 19	21 June 19	23 June 19		
TWCWC	13 July 19	13 July 19	14 July 19		
PLEON	17 July 19	24 July 19	15 July 19	Depth profiles, Secchi depth, light profile, plankton communities (Twin lakes only), PTOX screen*	Beth Norman Colleen Lawlor
TWCWC	17 Aug 19	17 Aug 19	18 Aug 19		

**conducted as part of the Miami University Pocono Lake HAB Survey.*

B. Summary of water quality

Table 2. Summary of Big Twin Lake in 2019.

	22 June	13 July	17 July	17 August
Thermally stratified?	YES*	YES*	YES	YES
Epilimnion depth (m)	6	4	3	4.5
Metalimnion depth (m)	>7	>7.5	9	8
Secchi depth (m)	2.5	2.2	2.5	2.5
Vertical extinction coefficient (k)	-	-	0.80	-
$Z_{10\%}$ (m)	-	-	2.88	-
$Z_{1\%}$ (m)	-	-	5.75	-
DO at max depth (mg/L)	2.89**	0.07**	0.71	0.21
Epilimnetic chlorophyll concentration (µg/L)	2.15	0.88	-	5.87
TN in the epilimnion (mg/L)	0.25	0.22	-	0.20
TP in the epilimnion (µg/L)	10.6	11.1	-	9.7†
TSI_{secchi}	46.8	48.6	46.8	46.8
$TSI_{chlorophyll}$	38.1	29.3	-	47.7
TSI_{TP}	37.7	38.3	-	36.4
Trophic classification* **	OLIGOTROPHIC	OLIGOTROPHIC	-	MESOTROPHIC
PTOX cyanobacteria found?	-	-	YES	-
Toxin testing recommended?	-	-	NO	-

**incomplete profile, **at deepest measured, not maximum depth, ***According to $TSI_{chlorophyll}$. Classification depends on TSI metric. †below the analytical detection limit.*

Table 3. Summary of Little Twin Lake in 2019.

	21 June	13 July	24 July	17 August
Thermally stratified?	YES*	YES*	YES	YES*
Epilimnion depth (m)	3.5	3.5	4	5
Metalimnion depth (m)	>8	>7.5	10	>9.5
Secchi depth (m)	3.4	3.5	3.5	2.2
Vertical extinction coefficient (k)	-	-	0.52	-
$Z_{10\%}$ (m)	-	-	4.46	-
$Z_{1\%}$ (m)	-	-	8.92	-
DO at max depth (mg/L)	5.17**	9.90**	0.67	0.49
Epilimnetic chlorophyll concentration (µg/L)	0.48	0.49	-	1.17
TN in the epilimnion (mg/L)	0.15†	0.18†	-	0.17†
TP in the epilimnion (µg/L)	7.7†	6.4†	-	7.2†
TSI_{secchi}	42.4	41.9	41.9	48.6
$TSI_{chlorophyll}$	23.4	23.7	-	32.1
TSI_{TP}	33.1	30.4	-	32.1
Trophic classification* **	OLIGOTROPHIC	OLIGOTROPHIC	-	OLIGOTROPHIC
PTOX cyanobacteria found?	-	-	YES	-
Toxin testing recommended?	-	-	NO	-

**incomplete profile, **at deepest measured, not maximum depth, ***According to $TSI_{chlorophyll}$. Classification depends on TSI metric. †below the analytical detection limit.*

Table 4. Summary of Walker Lake in 2019.

	23 June	14 July	15 July	18 August
Thermally stratified?	YES	PARTIAL	YES	YES*
Epilimnion depth (m)	2.5	2.5	2	3
Metalimnion depth (m)	6	6†	6	>6.5
Secchi depth (m)	1.4	1.2	1.1	1.25
Vertical extinction coefficient (k)	-	-	1.47	-
$Z_{10\%}$ (m)	-	-	1.56	-
$Z_{1\%}$ (m)	-	-	3.13	-
DO at max depth (mg/L)	0.06	0.17	0.85	0.13**
Epilimnetic chlorophyll concentration (µg/L)	3.32	4.22	-	5.02
TN in the epilimnion (mg/L)	0.30	0.36	-	0.44
TP in the epilimnion (µg/L)	18.0	22.3	-	23.0
TSI_{secchi}	55.2	57.3	58.6	56.8
$TSI_{chlorophyll}$	42.4	44.7	-	46.4
TSI_{TP}	45.2	48.2	-	48.7
Trophic classification* **	MESOTROPHIC	MESOTROPHIC	-	MESOTROPHIC
PTOX cyanobacteria found?	-	-	YES	-
Toxin testing recommended?	-	-	NO	-

**incomplete profile, **at deepest measured, not maximum depth, ***According to $TSI_{chlorophyll}$. Classification depends on TSI metric.*

II. 2019 Physical Profile Results

A. Temperature and Secchi Depth

All three lakes were thermally stratified although probe cable length prevented complete delineation of layers in some cases (Figures 1-2). June temperature was generally cooler compared to July and August by at least 3°C. Average epilimnetic temperatures of the three lakes were similar during the same sampling period. Deep water temperatures were warmer in Big Twin (average = 11.7°C) than in Little Twin (average = 6.3°C) and Walker (average = 8.6°C) during PLEON sampling in late July (only complete profile of all lakes).

Secchi depth ranged from 2.2-2.5 m in Big Twin, from 2.2-3.5 m in Little Twin, and from 1.1-1.4 m in Walker (Tables 2-4). Secchi depth can be used to calculate Carlson's Trophic State Index (TSI)[1]:

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

TSI is used to assign a trophic classification (Table 5). Average TSI_{Secchi} of Big Twin, Little Twin, and Walker in 2019 were 47.3, 43.7 and 57.0, respectively. Big and Little Twin are classified as mesotrophic while Walker is classified as eutrophic according to average TSI_{Secchi}.

Table 5. Trophic classification description

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

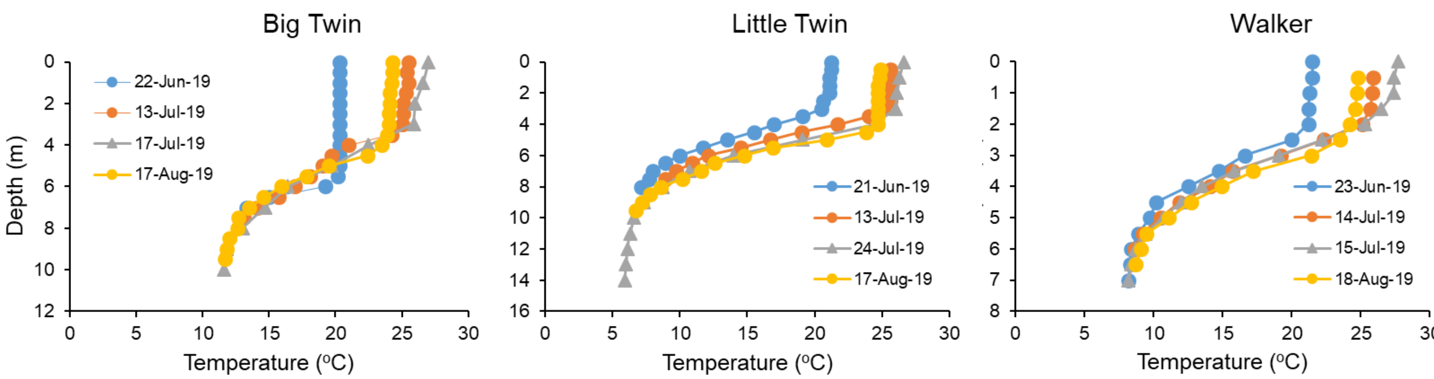


Figure 1. Temperature profiles of Twin and Walker lakes during 2019. Note differences in scale.

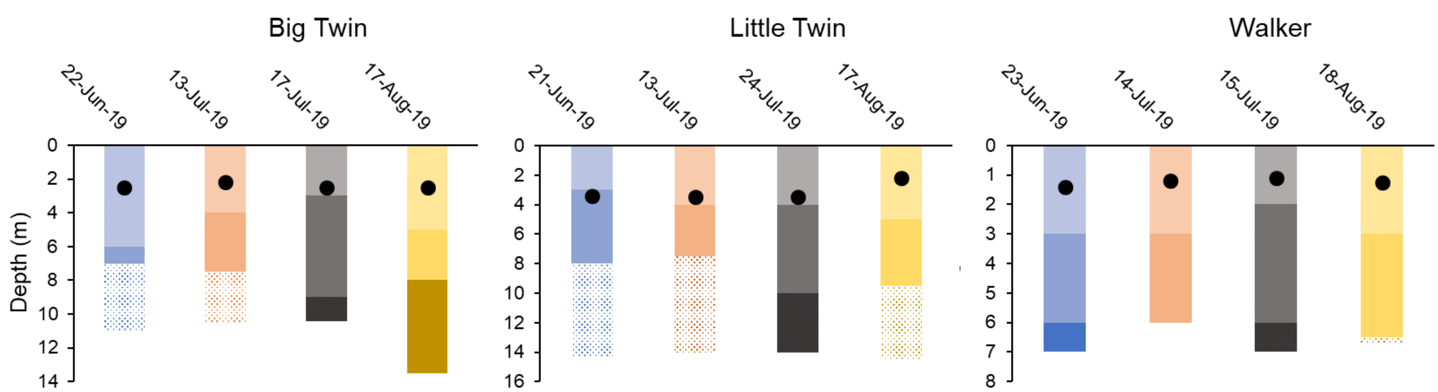


Figure 2. Stratification of Twin and Walker lakes during 2019. Note differences in scale. Bar shading denotes stratification. Closed circles show Secchi depth. Depths greater than cable length are hashed.

B. Dissolved Oxygen

Surface waters of the three lakes were oxygenated with DO concentration generally decreasing over the summer (Figure 3).

DO concentration fell below the 2 mg/L hypoxia threshold at 6 m in Big Twin, 9 m in Little Twin, and 2.5 m in Walker during the PLEON July sampling (only complete profile of Big and Little Twin). DO concentration in Walker Lake was consistently hypoxic below 4 m throughout the summer. Little Twin showed consistent metalimnetic DO maxima through the summer suggesting high algal abundance in the middle waters. Peak DO concentration ranged from 11.5-13.4 mg/L at a depth of 6-6.5 m.

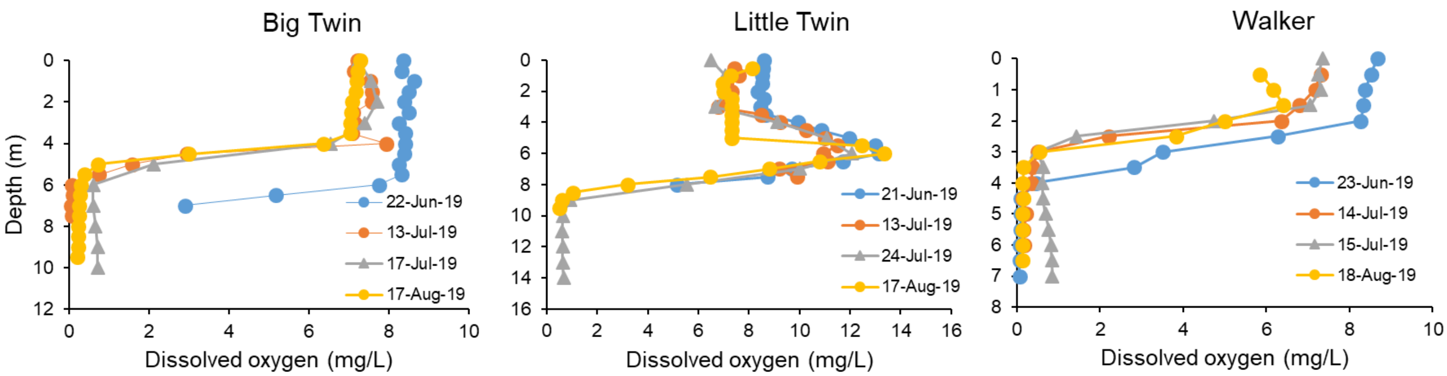


Figure 3. Dissolved oxygen profiles of Twin and Walker lakes in 2019. Note differences in scale.

C. Conductivity

Conductivity was highest in Little Twin (summer average at 1 m = 137 µS/cm), followed by Big Twin (70 µS/cm) and Walker (66 µS/cm; Figure 4). Conductivity was greatest in August in all three lakes (Figure 4). This increase could be due to a seasonal decline in precipitation and/or an increase in evaporation, concentrating lake water. Conductivity generally increased with depth across all months, with the exception of the PLEON July sampling of Big and Little Twin which showed higher conductivity at the surface.

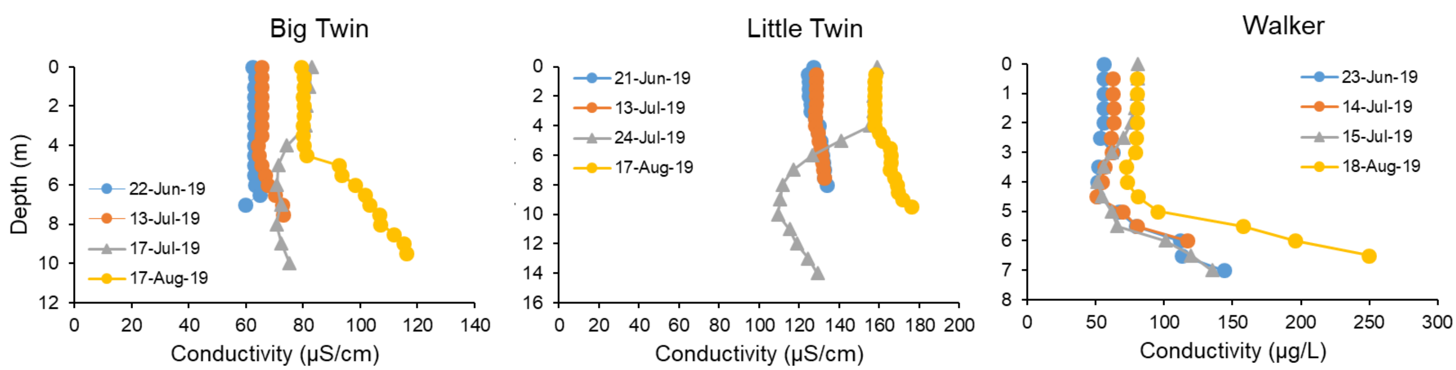


Figure 4. Conductivity profiles of Twin and Walker lakes during 2019. Note differences in scale.

D. pH

pH depth and seasonal patterns differed among lakes (Figure 5). In Big Twin, pH of the epilimnion in June was more acidic than later in the summer and pH generally declined with depth during all months except in June where pH at the deepest depth measured was >3 units greater than the rest of the water column. pH of Little Twin ranged from 6.21-9.79 and pH patterns with depth varied greatly among sampling dates (e.g. minimum or maximum pH recorded between 4-8 m depending on month). Walker had the most consistent pH profiles among sampling dates. pH in this lake generally decreased through depth until ~5 m after which pH increased with depth.

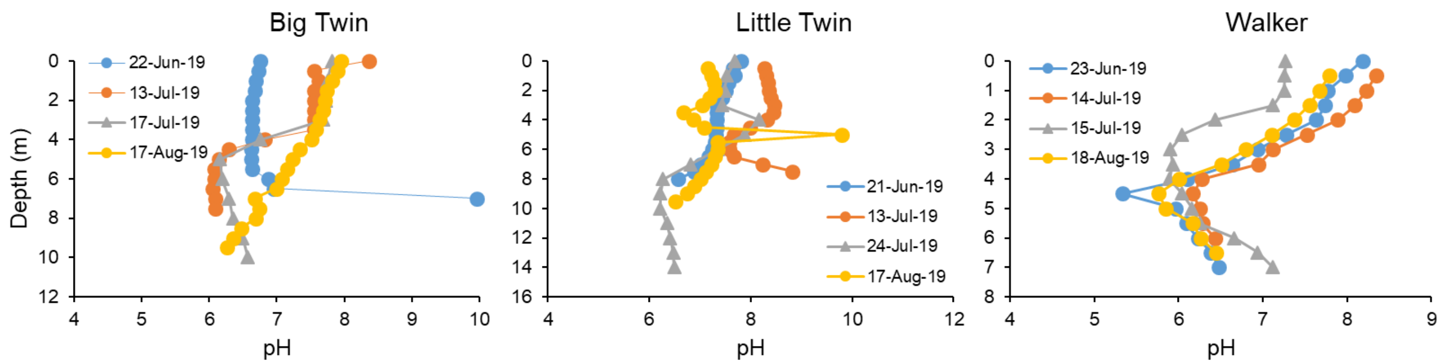


Figure 5. pH profiles of Twin and Walker lakes during 2019. Note differences in scale.

E. Total Dissolved Solids

Total dissolved solids (TDS) concentration was greatest during August (Figure 6), particularly in Big Twin and Little Twin. As with conductivity, the increase in TDS concentration could be due to changes in precipitation and/or evaporation. TDS concentration generally increased with depth in all lakes likely due to increased influence of sediments. Little Twin had the greatest TDS concentration in the surface waters followed by Walker and Big Twin.

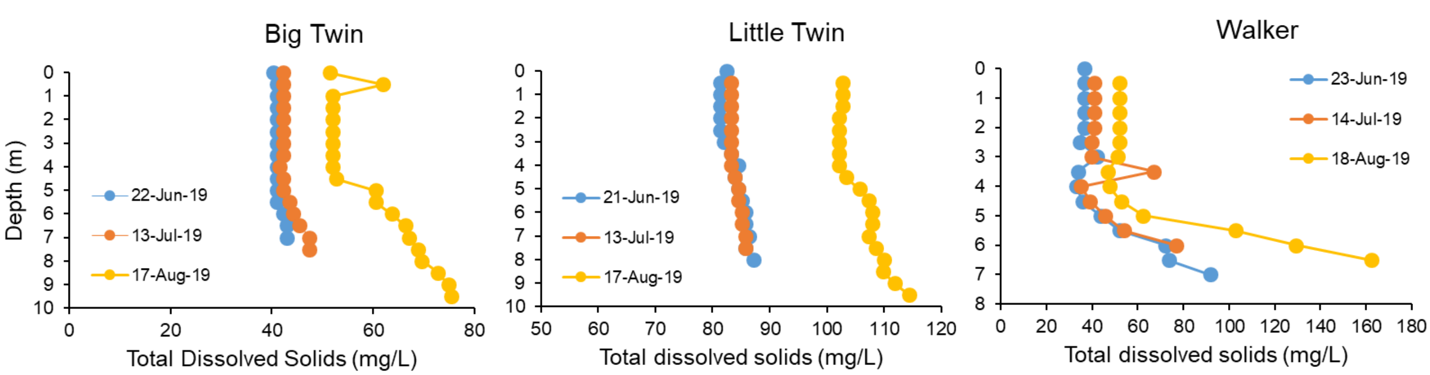


Figure 6. Total dissolved solids profiles of Twin and Walker lakes during 2019. Note scale differences.

F. Light

Dissolved and particulate material affect the rate at which light intensity attenuates with depth. Light intensity declines exponentially (Figure 7) 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). Wavelengths between 400-700 nm (photosynthetically active radiation; PAR) are used for photosynthesis.

Little Twin was the clearest of the lakes followed by Big Twin and Walker (Table 6).

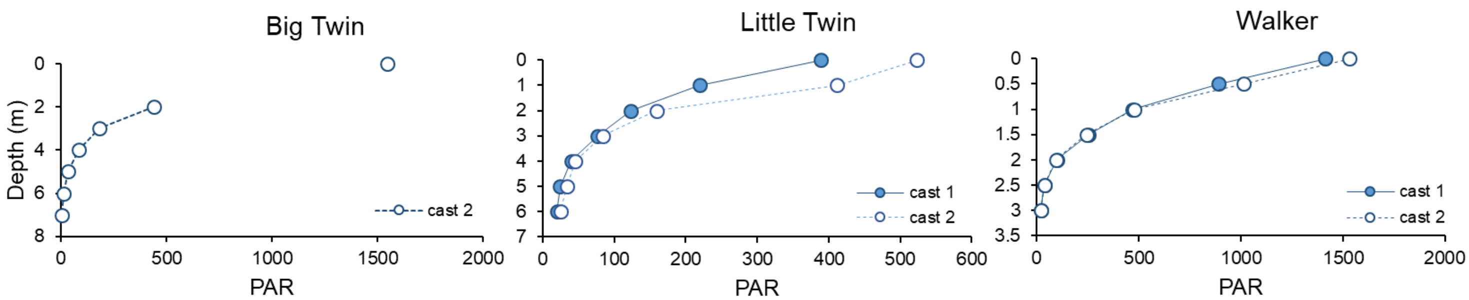


Figure 7. Depth profiles of PAR in Twin and Walker lakes in July 2019. Note differences in scale.

Table 6. Light intensity parameters calculated from PAR profiles in Twin and Walker lakes in July 2019. Parameters were calculated from the Cast 1 in Little Twin and Walker and Cast 2 in Big Twin.

	k	$Z_{10\%}$	$Z_{1\%}$
Big Twin	0.80	2.88	5.75
Little Twin	0.52	4.46	8.92
Walker	1.47	1.56	3.13

III. Chlorophyll Results

Chlorophyll *a* (chla) concentration at 0.5 m was greatest during August in all the lakes (Figure 8). Chla concentration increased over time in Little Twin and Walker but was lowest in July in Big Twin.

Walker lake had the greatest chla concentration in June and July while Big Lake had the greatest chla concentration in August.

The composite sample had lower chla concentrations than the 0.5 m in June and July in Big Twin and Walker indicating a concentration of algae at the surface. In contrast, composite samples from Little Twin consistently contained more chla than the 0.5 m samples indicating a concentration of algae at deeper depths, perhaps to avoid UV radiation exposure in this clear lake. The metalimnetic DO maxima observed in Little Twin also suggest an abundance of algae at middle depths.

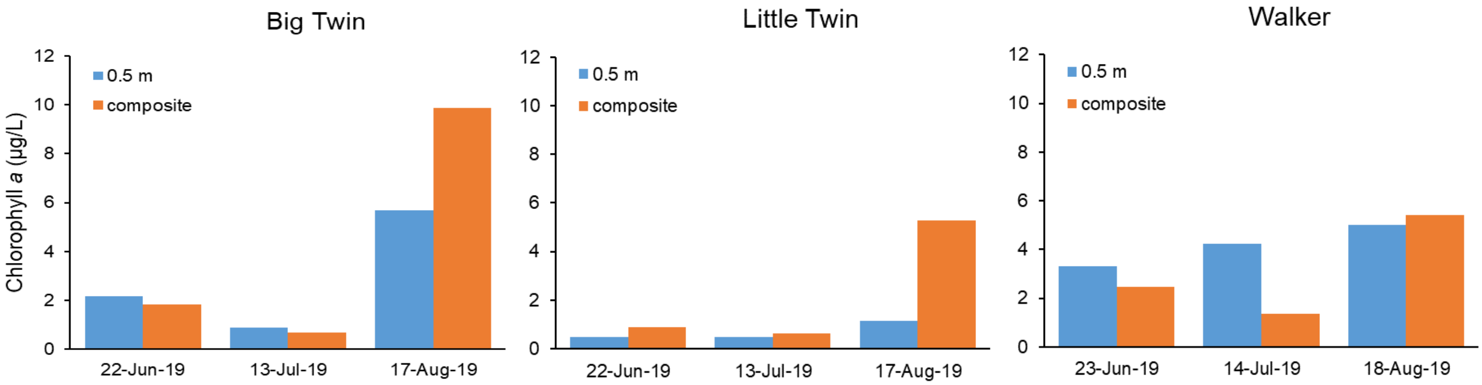


Figure 8. Chlorophyll* a *concentration in Twin and Walker lakes in 2019. Bars are single samples.

TSI can be calculated from chla concentrations measured at 0.5 m according to the following equation[1]:

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

The average $TSI_{chlorophyll}$ of Big Twin, Little Twin, and Walker were 38.4, 26.4, and 44.5, respectively. According to these values, Big Twin and Little Twin are classified as oligotrophic and Walker is classified as mesotrophic (Table 5).

IV. Nutrient Results

A. Total nitrogen

Total nitrogen concentration (TN) in Big Twin and Little Twin remained fairly constant through the summer in the surface waters with a summer average of 0.22 mg/L and 0.17 mg/L (below the limit of detection), respectively (Figure 9). TN of composite sample were generally greater for both lakes, indicating nitrogen-rich deep waters. This was particularly evident in Little Twin during the August sampling when TN of the composite sample was over twice that of the surface sample.

Surface samples from Walker had more TN than Big Twin and Little Twin with a summer average of 0.37 mg/L (Figure 9). TN of Walker composite samples was also greater than surface samples, indicating nitrogen-rich deep waters in this lake as well. TN in both sample types increased over the course of the summer in Walker.

TN concentration in all three lakes was below the Penn State Extension office suggested 3 mg/L threshold for nutrient pollution[2]

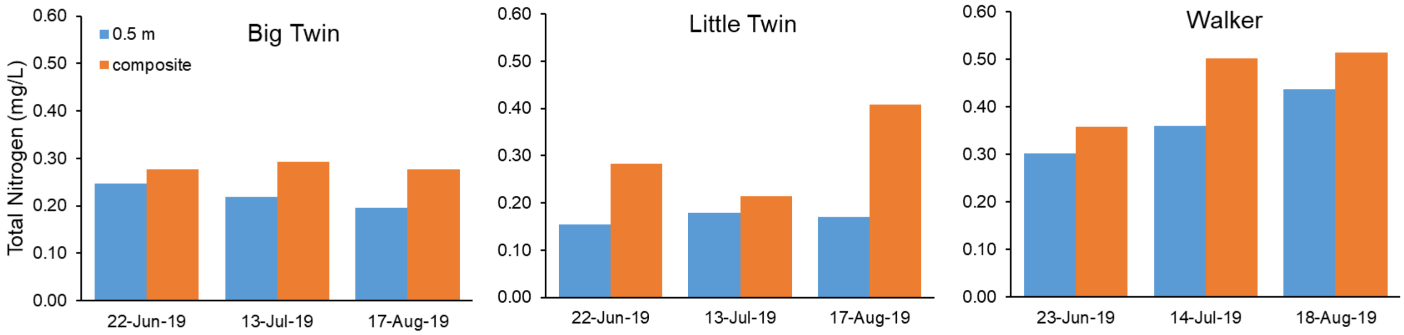


Figure 9. Total nitrogen concentration in Twin and Walker lakes in 2019. Bars are single samples. The analytical detection limit is 0.20 mg/L.

B. Total phosphorus

Total phosphorus concentration (TP) in Big Twin and Little Twin was relatively stable through the summer in the surface waters with a summer average of 8.9 µg/L and 7.1 µg/L, respectively (Figure 10). Both values are below the limit of detection. Composite samples were more concentrated than the surface, indicating more TP in deep waters.

Walker had more TP than the other lakes with a summer average of 21.1 µg/L (Figure 10). Walker composite samples had more TP than surface samples, indicating more TP in deep waters in this lake as well. Walker TP generally increased over the summer. TP concentration in all three lakes was below the Penn State Extension office suggested 25 µg/L threshold for nutrient pollution[2]

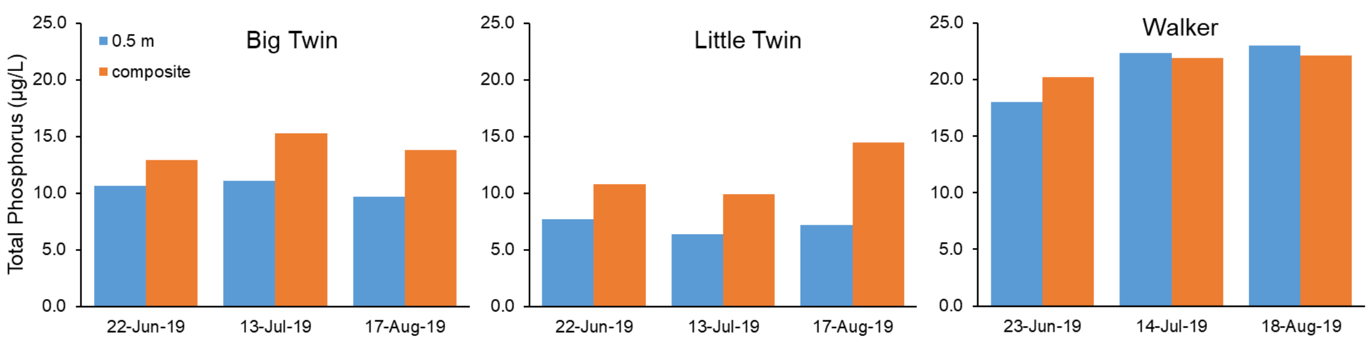


Figure 10. Total phosphorus concentration in Twin and Walker lakes in 2019. Bars are single samples. The analytical detection limit is 10 µg/L.

Trophic State Index can be calculated from TP concentrations at 0.5 m as[1]:

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

Average TSI_{TP} of Big Twin, Little Twin, and Walker was 35.2, 31.9, and 47.4, respectively. The Twin lakes are classified as oligotrophic and Walker as mesotrophic (Table 5).

V. Plankton Communities

A. Zooplankton

Zooplankton communities in Big Twin and Little Twin were numerically dominated by rotifers, followed by copepods and cladocerans (Table 7). Copepods were the dominant group by mass, followed by cladocerans and rotifers. Although not numerous, Chaoboridae, or predatory phantom midges, made up ~7% and ~6% of zooplankton biomass in Big Twin and Little Twin, respectively.

The average zooplankton richness was 13 in both lakes. The Shannon-Wiener diversity index was 0.73 in Big Twin and 0.71 in Little Twin.

Table 7. Density and biomass of zooplankton taxa collected from Big Twin and Little Twin on 17 July 2019 and 24 July 2019, respectively. Values from Big Twin are averages of 2 replicates.

	Big Twin				Little Twin			
	Density (cells/ml)	Relative density (%)	Biomass (µg/ml)	Relative biomass (%)	Density (cells/ml)	Relative density (%)	Biomass (µg/ml)	Relative biomass (%)
PROTOZOA	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
ROTIFERA	**90.4**	**72.9**	**9.9**	**9.6**	**88.0**	**71.5**	**9.1**	**9.6**
Anuraeopsis	0.3	<1	0.01	<1				
Asplanchna	2.5	2.0	2.5	2.4	1.6	1.3	1.6	1.7
Conochilus	5.6	4.5	0.2	<1	1.1	<1	0.04	<1
Kellicottia					1.1	<1	0.04	<1
Keratella	39.2	31.6	3.5	3.4	60.5	49.1	5.4	5.8
Polyarthra	38.6	31.1	3.5	3.4	19.4	15.8	1.7	1.9
Synchaeta	0.3	<1	0.0	<1				
Trichocerca	3.9	3.2	0.2	<1	4.3	3.5	0.2	<1
COPEPODA	**26.6**	**21.4**	**64.7**	**62.7**	**29.7**	**24.1**	**68.4**	**72.6**
Cyclops group	2.2	1.8	5.5	5.3	3.8	3.1	9.2	9.8
Mesocyclops	3.4	2.7	4.2	4.1	5.9	4.8	7.4	7.9
Diaptomus group	0.3	<1	0.1	<1	0.5	<1	0.3	<1
nauplii	20.7	16.7	54.9	53.2	19.4	15.8	51.5	54.6
CLADOCERA	**7.0**	**5.6**	**21.5**	**20.9**	**5.4**	**4.4**	**11.4**	**12.1**
Bosmina	2.2	1.8	2.8	2.7	1.6	1.3	1.6	1.7
Ceriodaphnia	4.5	3.6	16.4	15.9	3.8	3.1	9.8	10.4
Holopedium	0.3	<1	2.3	2.3				
OTHER	**0.01**	**<1**	**7.0**	**6.8**	**0.01**	**<1**	**5.4**	**5.7**
Chaoboridae	0.01	<1	7.0	6.8	0.01	<1	5.4	5.7
TOTAL	**124.1**		**103.2**		**123.1**		**94.3**	

B. Phytoplankton

Cyanophyta, or cyanobacteria, were the numerically dominant algal group in both Big Twin and Little Twin (76% and 65% of the communities, respectively), followed by Chlorophyta (green algae) in Big Twin and by Chrysophyta (golden-brown algae) in Little Twin (Table 8). Total abundance and within-group abundance was higher in Big Twin compared to Little Twin with the exception of Bacillariophyta (diatoms) and Chrysophyta. Phytoplankton biomass in both lakes was spread more evenly among groups although Cyanophyta was still dominant by mass.

Within the Cyanophyta, filamentous nitrogen fixers were the most abundant in both lakes. Members of this group were identified as *Dolichospermum*, a genus known to be potentially toxigenic.

Table 8. Density and biomass of phytoplankton taxa collected from Big Twin and Little Twin on 17 July 2019 and 24 July 2019, respectively. Values are averages of 2 replicates.

	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	**22.1**	**<1**	**22.9**	**1.7**	**134.8**	**7.1**	**115.64**	**16.0**
Centric Diatoms	13.1	<1	15.7	1.2	19.5	1.0	23.4	3.2
Araphid Pennate Diatoms	9	<1	7.2	<1	115.3	6.0	92.24	12.8
CHLOROPHYTA	**648**	**18.0**	**387.9**	**29.0**	**94.75**	**5.0**	**80.315**	**11.1**
Coccoid/Colonial Chlorophytes	369	10.3	110.7	8.3	45.15	2.4	4.515	<1
Desmids	279	7.8	277.2	20.8	49.6	2.6	75.8	10.5
CHRYSOPHYTA	**99**	**2.8**	**217.6**	**16.3**	**400.8**	**21.0**	**234.8**	**32.5**
Flagellated Classic Chrysophytes	94.1	2.6	216.8	16.2	400.8	21.0	234.8	32.5
Tribophytes/Eustigmatophytes	5.0	<1	0.7	<1	0		0	
CRYPTOPHYTA	**80.1**	**2.2**	**66.4**	**5.0**	**14.2**	**<1**	**2.84**	**<1**
CYANOPHYTA	**2713.5**	**75.5**	**449.5**	**33.7**	**1242**	**65.2**	**248.4**	**34.4**
Filamentous Nitrogen Fixers	2223	61.9	444.6	33.3	1242	65.2	248.4	34.4
Filamentous Non-Fixers	490.5	13.6	4.9	<1	0		0	
EUGLENOPHYTA	**8.1**	**<1**	**5.3**	**<1**	**0**		**0**	
PYRRHOPHYTA	**23.0**	**<1**	**185.9**	**13.9**	**19.5**	**1.0**	**40.95**	**5.7**
TOTAL	**3593.7**		**722.9**		**1906.1**		**722.9**	

VI. PTOX Cyanobacteria Screen

Surface samples collected from 2 locations within Little Twin and Walker and 1 location with in Big Twin were screened by GreenWater Laboratories for potentially toxigenic (PTOX) cyanobacteria (Appendix II). This screening was not included in the PLEON package but was conducted as a part of the Pocono Lakes HABs Survey led by Miami University in collaboration with PLEON.

Dolichospermum and Aphanizomenon species were found in small numbers in Twin and Walker lakes (Table 9). Due to this low abundance, toxin analysis was not recommended. Microcystin concentration was measured in the field at the time of sample collection using semi-quantitative test strips. Toxin tests were negative (<10 µg/L) for each sample.

Table 9. Summary of PTOX screens of Twin and Walker lakes in July 2019.

		Observed Taxa	Count*	Potential Toxins**	Greenwater recommendation
Big Twin	Center (A)	*Dolichospermum sp.*	<10	MIC, SAX, ANA, CYL	Toxin testing not recommended
Little Twin	Center (A)	*Dolichospermum sp.*	3	MIC, SAX, ANA, CYL	Toxin testing not recommended
	Near Shore (B)	None observed	-	-	-
Walker	Center (A)	None observed	-	-	-
	Near Shore (B)	*Aphanizomenon sp.*	1	MIC, SAX, ANA, CYL	Toxin testing not recommended

**Counts are filaments/ml. Counts should not be used for thresholds but for assessing qualitative changes within a site. **SAX = saxitoxins, CYL = cylindrospermopsin, ANA = anatoxin-a, MIC = microcystins*

VII. Historical Context: Twin and Walker Lakes Over Time

A. Description of historical dataset

2019 was the first year of PLEON monitoring of the Twin and Walker lakes. Data from 2014-2018 were provided by the TWCWC in the form of yearly “state of the lake” reports by FX Browne.

B. Temperature and dissolved oxygen

July surface temperature has been variable since 2014 in all three lakes (Figure 11). Surface temperature in all lakes shows a general increase over time. In recent years, temperature patterns among lakes has diverged, particularly in Big Twin during 2018.

This could suggest a local driver of lake temperature such as the influence of a spring or stream input or may reflect a difference in sampling time. Continued monitoring will determine if surface temperatures continue to diverge.

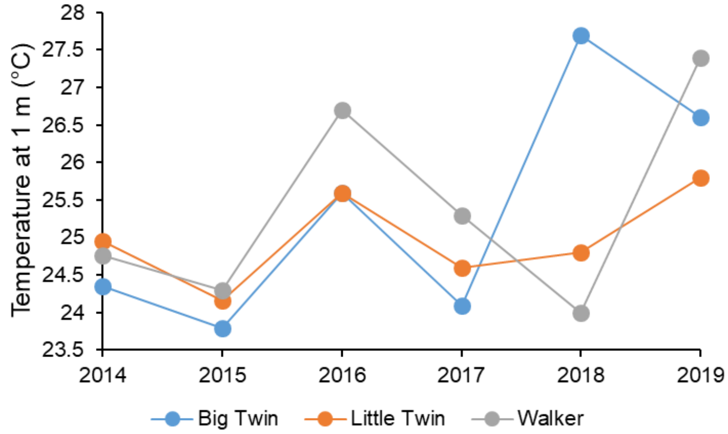


Figure 11. Surface temperatures (1 m) measured in July in Twin and Walker lakes since 2014.

Dissolved oxygen concentrations in the hypolimnion are important because of the potential for nutrient regeneration from sediments during periods of anoxia as well as oxygen stress for fish and other lake organisms. Hypolimnetic oxygen data are not available for Big Twin and Little Twin. Since 2014, Walker has consistently developed a hypoxic/anoxic hypolimnion (Table 10). The depth of oxygen depletion (defined as <2.0 mg/L) has ranged from 4-2.5 m since 2014.

Table 10. Hypolimnetic dissolved oxygen trends in Walker Lake since 2014.

	depth of oxygen depletion (m)	DO at this depth (mg/L)
2014	4	1.23
2015	3	1.82
2016	3	0.63
2017	3	1.03
2018	3	0.03
2019	2.5	1.43

C. Secchi depth

Little Twin has been the most transparent of the three lakes since 2014, followed by Big Twin and Walker (Figure 12). Secchi depth has remained fairly consistent in all three lakes since 2014. According to TSI_{Secchi}, Little Twin has been oligo-mesotrophic, Big Twin has been mesotrophic, and Walker has been eutrophic since 2014.

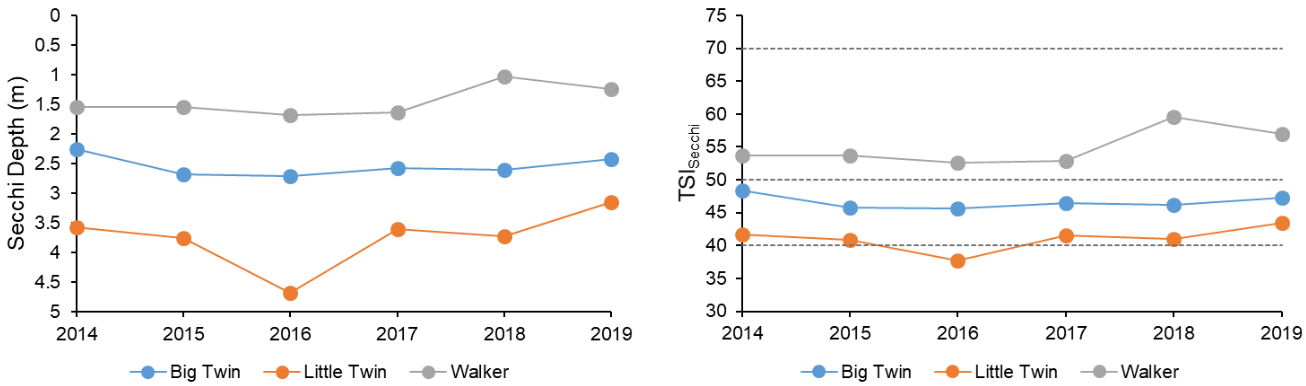


Figure 12. Average Secchi depth (left) and TSI_{Secchi} (right) in Twin and Walker lakes since 2014. Horizontal dashed lines in right panel show boundaries of oligotrophic (<40), mesotrophic (40-50), eutrophic (50-70), and hypereutrophic (>70) classifications.

D. Chlorophyll

Patterns of chlorophyll a, a proxy for algal biomass, have generally been similar over time in all three lakes (Figure 13). Little Twin has been consistently less productive than Big Twin and Walker since 2014. Walker has been the most productive in all years except 2017. Productivity in Big Twin and Little Twin has been declining since 2017. According to $TSI_{chlorophyll}$, all three lakes were mesotrophic from 2014-2016. Big Twin and Walker have periodically been classified as eutrophic since 2016.

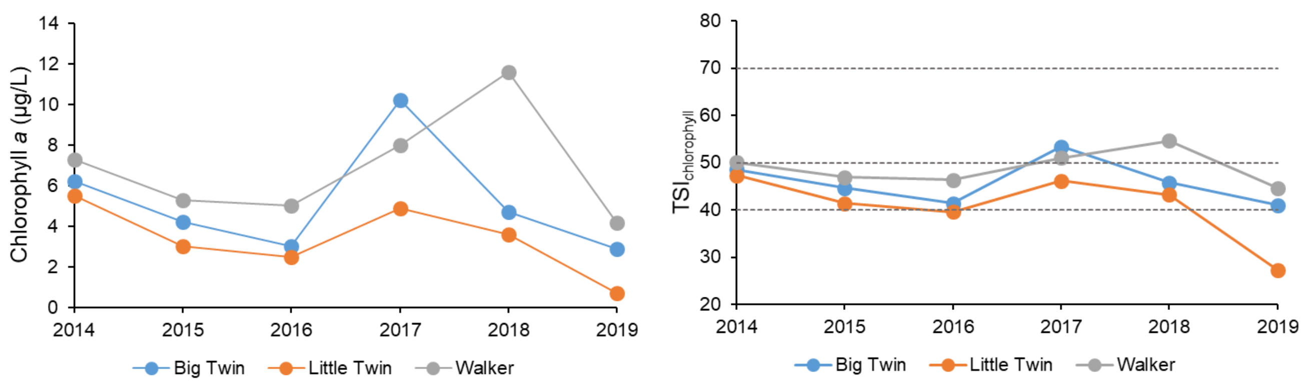


Figure 13. Average chlorophyll** a **concentration (left) and $TSI_{chlorophyll}$ (right) in Twin and Walker lakes since 2014. Horizontal dashed lines in right panel show boundaries of oligotrophic (<40), mesotrophic (40-50), eutrophic (50-70), and hypereutrophic (>70) classifications.

E. Nutrients

The temporal pattern of TP concentration is similar among all lakes with a peak in 2016 (Figure 14). TP concentration has generally been least in Little Twin and greatest in Walker since 2014. Since 2017, TP concentrations in Big Twin and Little Twin have converged while TP in Walker has been roughly 2x that of the other lakes. According to TSITP, Walker has remained mesotrophic over the sampling period while Big Twin and Little Twin shifted from mesotrophy to oligotrophy in 2018.

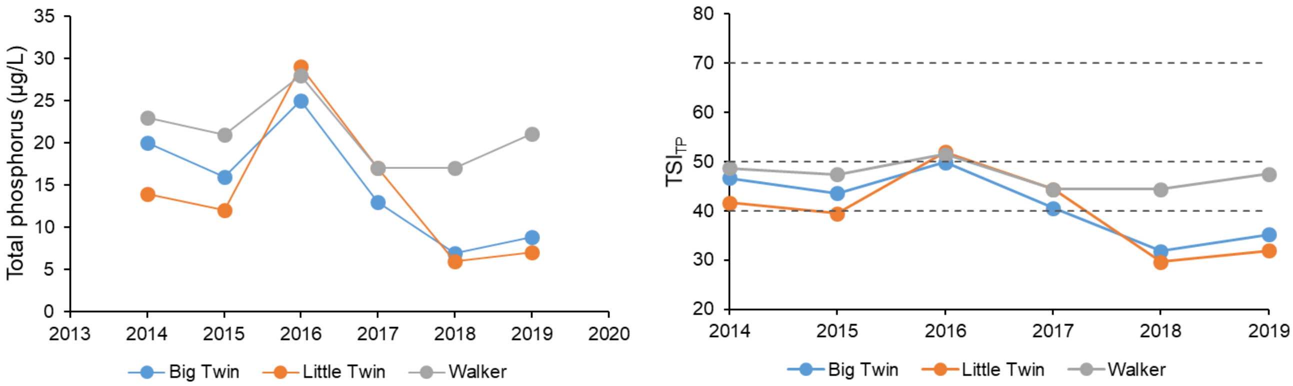


Figure 14. Average TP concentration (left) and TSI_{TP} (right) in Twin and Walker lakes since 2014. Horizontal dashed lines in right panel show boundaries of oligotrophic (<40), mesotrophic (40-50), eutrophic (50-70), and hypereutrophic (>70) classifications.

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

A. Description of PLEON Lakes

As of 2019, the PLEON dataset consists of 18 lakes in Pike, Wayne, and Monroe Counties. Lakes range from ~80,000-1,130,000 m^2 (average = 370,000 m^2) in surface area, ~1,400-8,000 m (average = 3,000 m) in shoreline and ~2-23 m (average = 7.8 m) in maximum depth (Figure 15).

Big Twin is the largest PLEON lakes in surface area and is above the PLEON average shoreline and maximum depth. In contrast, Little Twin is below the PLEON average surface area and shoreline but deeper than the PLEON average maximum depth. Walker is slightly above the PLEON average in regards to surface area and shoreline and slightly shallower than the PLEON average maximum depth.

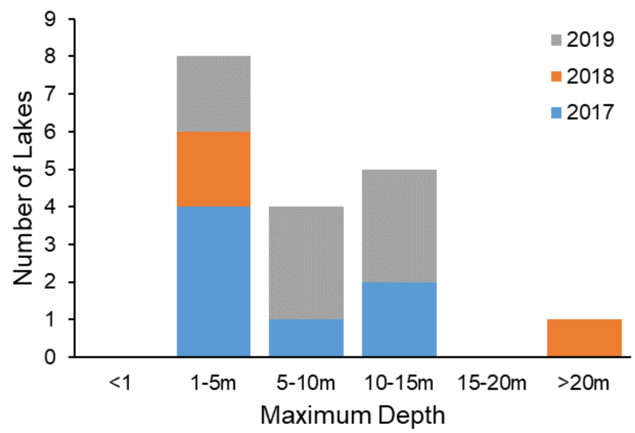


Figure 15. Distribution of PLEON lakes according to maximum depth (m). Colors denote when PLEON monitoring began.

B. Trophic classification

Eutrophic, mesotrophic, and oligotrophic lakes are represented in the PLEON dataset (defined according to TSI_{Secchi}). Most PLEON lakes are either eutrophic (10 lakes) or mesotrophic (6 lakes; Figure 16).

Big and Little Twin are classified as mesotrophic. The three other PLEON lakes in the same maximum depth category (10-15 m) are glacial lakes. Two are oligotrophic and the third is mesotrophic. Walker lake is classified as eutrophic. Two other PLEON lakes in the same maximum depth category (5-10 m) have lakeside communities and are eutrophic or mesotrophic. Another is an isolated mesotrophic glacial lake.

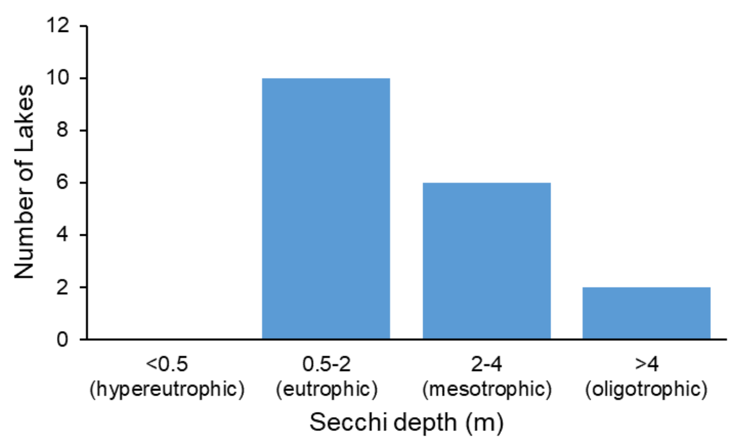


Figure 16. Distribution of PLEON lakes according to the latest recorded summer Secchi depth. Note that some lakes are monitored every other year.

C. Nutrients

PLEON lakes are evenly distributed across a range of epilimnetic TN and TP concentrations (Figure 17). Big and Little Twin are within the lowest group for both nutrients while Walker is within the 2nd group for both nutrients.

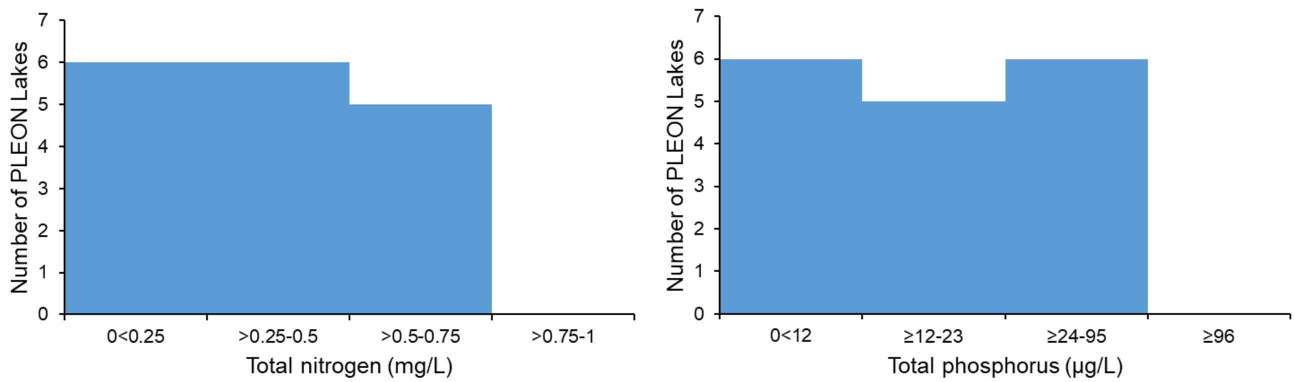


Figure 17. Distribution of PLEON lakes according to the latest recorded summer TN and TP concentration. Note that some lakes are monitored every other year.

D. PTOX Cyanobacteria

PTOX screens of Twin and Walker lakes showed low abundance of PTOX cyanobacteria in July 2019 (Table 11). Both genera found (*Aphanizomenon* and *Dolichospermum*) were found in several other PLEON lakes.

Table 11. Number of PLEON lakes with PTOX taxa. Yellow fill indicates Walker is in that category while green fill indicates Big Twin and Little Twin are in that category.

	2017				**2018**				**2019**			
	M	**J**	**J**	**A**	**J**	**J**	**A**	**S**	**M**	**J**	**J**	**A**
Aphanizomenon spp.	1	0	1	3	1	1	1	1	1	1	2	2
Chrysosporum spp.	0	1	1	0	0	1	2	0	1	1	3	2
Cuspidothrix spp.	1	1	0	0	0	1	1	1	0	0	1	1
Dolichospermum spp.	1	1	1	3	1	1	4	1	1	1	6	2
Microcystis spp.	1	1	2	3	1	0	2	0	0	0	2	1
Nostocalean genera	0	0	1	0	1	1	0	0	0	0	0	0
Planktothrix sp.	0	1	1	0	0	0	0	0	0	0	0	0
Woronichinia naegeliana	0	0	0	1	0	0	0	0	0	0	2	1
PTOX taxa not observed	0	0	2	0	0	0	2	0	0	0	0	2
# of lakes screened	**1**	**2**	**5**	**4**	**2**	**2**	**6**	**1**	**1**	**1**	**8**	**4**

PLEON has conducted a total of 94 PTOX screens since May 2017. Based on the recommendation of Greenwater Laboratories, many of these screens have been tested for specific toxins (Table 9). Microcystin/nodularins are the only toxins that have been found above the minimum detection limits in PLEON lakes. However, it is important to note that recommended testing has often been declined. None of the Twin and Walker lake samples were tested for toxins through Greenwater Labs as per Greenwater recommendations.

Table 9. Summary of PTOX screens tested for toxin concentration across PLEON lakes (2017-19).

Toxin	# recommended for testing	# tested	# < MDL*	# ≥ MDL*	Mean concentration (ng/mL)	Range (ng/mL)
microcystins/nodularins	29	24	12	12	15.3	0.16-129
cylindrospermopsin	22	15	15	0	-	-
anatoxin-a	20	16	16	0	-	-
saxitoxin	20	16	16	0	-	-

**minimum detection limits*

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

Several findings from the Twin and Walker lakes 2019 monitoring program should be highlighted:

1. Trophic classification depended on the metric used to calculate TSI. A comparison of TSI_{Secchi} and $TSI_{chlorophyll}$ shows that Secchi depth was more shallow than predicted by chlorophyll concentrations. This is not unusual as Secchi depth and chlorophyll are not measuring the same thing. Secchi depth is an estimate of water transparency while chlorophyll concentration quantifies algal abundance. These two variables are often, but not always, correlated. Dissolved and particulate materials other than algae can decrease transparency and therefore affect Secchi depth, resulting in a shallower Secchi depth (and greater TSI_{secchi}) than predicted by chlorophyll concentrations. $TSI_{chlorophyll}$ is a more direct measure of lake productivity.

2. Deep water hypoxia/anoxia was observed in all lakes. It is common for oxygen concentration to decline in the hypolimnion due to the lack of photosynthesis coupled with decomposition in the sediments. Anoxia in deep waters is important because biochemical processes change when oxygen is absent. Specifically, nutrients bound in the sediments are released under anoxic conditions. Released nutrients can fuel algal production when deep waters mix with surface waters during lake turnover.

Substantial increases in the amount of time anoxia is present and the depth of the anoxic layer can increase the amount of nutrients released from the sediments, posing a potential management concern. The duration and depth of anoxia can also affect some fisheries as many fish species require cool, well oxygenated waters to thrive. A recent analysis of long term oxygen data in 2 Pocono lakes suggest that deep water oxygen concentrations have been declining over the past several decades[3]. Monitoring of deep water oxygen concentrations is needed to determine if Twin and Walker lakes are following this pattern.

The current TWCWC monitoring program is developing this record in Walker Lake. Walker Lake has consistently had an oxygen depleted hypolimnion since 2014. The depth at which depletion occurs (<2 mg/L) has been between 2.5-4 m. It is too early to determine if the shift from anoxia at 3 m in 2018 to 2.5 m in 2019 is the beginning of a trend of a shallowing of the anoxic layer.

The length of the TWCWC YSI probe cable is insufficient to capture oxygen dynamics in the Twin lakes. PLEON's cable is long enough but TWCWC may want to consider investing in a longer cable in order to obtain complete oxygen profiles over the entire summer. Another option is to invest in oxygen data loggers that would be deployed just above the sediments and would record oxygen concentration continuously. The advantage to this system is the continuous data record (every hour, 24 hours a day) while the disadvantage is the data is from a single depth.

3. Little Twin Lake has high conductivity and TDS. This pattern has been fairly consistent since 2014 (Appendix VI). Conductivity is the ability of water to conduct electricity and is a measurement of the amount of dissolved ions, or charged particles, in the water. Sources of dissolved ions include geology and runoff. High conductivity can also be a result of septic inputs to a lake. Little Twin and Big Twin likely have similar geology, so the comparatively high conductivity of Little Twin (almost double that of Big Twin) is unexpected. The impacts of septic inputs may be diluted by the large size of Big Twin while exaggerated in Little Twin.

There were also interesting seasonal patterns of conductivity and TDS in all three lakes. Both variables were substantially higher in August compared to June and July. As previously noted, this may be due to a concentration of lake water caused by a combination of increased temperatures and low precipitation. It also may be a result of increased lake usage during late summer, which would presumably coincide with increased septic use and potentially leakage. Comparisons of early and late summer conductivity and TDS since 2014 does not show this seasonal increase consistently. There were some discrepancies between the July patterns recorded by PLEON and TWCWC. They may be due to probe differences or the fact that the profiles were conducted on different days.

4. Potentially toxigenic cyanobacteria were present in Twin and Walker lakes. The PTOX screens and in-field microcystin testing described here were conducted as part of the Pocono Lakes HABs Survey, a collaboration between Miami University's Lauren Knose and PLEON. The results of these screens show low abundances of PTOX taxa in Twin and Walker lakes.

However, the phytoplankton community analysis found *Dolichospermum* made up >60% of the algal community in Big Twin and Little Twin. *Dolichospermum* is capable of producing several cyanotoxins, including microcystin, saxitoxin, anatoxin, and cylindrospermopsin. The phytoplankton community analysis was conducted on a composite sample consisting of water collected from 0.5, 2, 4, and 6 m while the PTOX screen was conducted on water collected from 0.5 m. The abundance of *Dolichospermum* in the community analysis compared to the PTOX screen indicates that the cyanobacteria were deeper in the water column in these lakes.

Cyanobacteria are present in most lakes. However, an abundance of PTOX taxa below the surface means there is the potential for blooms to develop as cyanobacteria change position. There is also the potential for below surface blooms that can be difficult to detect but may still be harmful.

It is important to note that these results pertain only to the sampling date. Algal communities are very dynamic and their abundance can change quickly, sometimes in a matter of hours. The fact that PTOX taxa were present suggests that the potential exists for harmful algal blooms in these lakes. TWCWC may want to consider a comprehensive HABs monitoring plan.

5. Phosphorus concentration may be declining (but with lots of variation). There appears to be an overall decrease in TP concentration in Twin and Walker lakes since 2014. However, there is also a lot of interannual variation and 6 years is not a long data set. Chlorophyll also seems to be generally declining (although also with a lot of variation). Interestingly, peak chlorophyll measurements did not occur at the same time as peak TP measurements within this dataset. Continued monitoring is needed before statistically significant trends can be identified.

Hypolimnetic nutrient concentrations are not being measured in Big Twin and Little Twin according to the current sampling regimen. Nutrients, phosphorus in particular, can be released from the sediments under anoxic conditions (see Point 2 above). These nutrients are then brought to the surface during periods of lake mixing where they can fuel algal production. Nutrient regeneration can be an important nutrient source in lakes. The fact that composite samples contained more nutrients than the surface samples does indicate that there are more nutrients in the deeper waters, but dilution with surface water makes interpreting these data difficult. TWCWC may wish to consider collecting hypolimnion samples in the future to capture changes in nutrient regeneration.

APPENDIX I: Description of Field Sampling Methods

A. Physical Profiles

PLEON measured temperature, dissolved oxygen, conductivity, and pH using a handheld YSI Professional Plus multiparameter instrument fitted with a polarographic dissolved oxygen probe and a pro series pH probe. PLEON probes were calibrated in early June 2019. 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. A LiCOR spherical quantum sensor (model LI-193) was used to quantify irradiance.

B. Chlorophyll

Chlorophyll a concentration was measured using the method developed by Robert Moeller and currently used by the Williamson Lab. Water samples were collected by TWCWC from 0.5 m and from several depths and mixed into a composite sample. Samples were kept cold until filtered. For each replicate, a known volume was filtered onto a glass fiber filter with nominal pore size of 0.7 µm using a vacuum pump. Filters were frozen until extraction. Pigments were extracted from filters with 10 ml of a 5:1 acetone:methanol solution. The extraction took place over 48 hours at -20°C with a 2-minute heating step (60°C) after 24 hours. Chlorophyll concentration of the extractant was determined via fluorometry (Turner Designs 10AU fluorometer) and corrected for phaeophyton according to EPA method 445.0.

C. Nutrients

Two water samples were collected by TWCWC from 0.5 m and from several depths and mixed into a composite sample. Water samples were collected in acid washed bottles and kept cold until return to the lab. A 60 ml subsample of each replicate was acidified using concentrated sulfuric acid. Acidified samples were kept cold (4°C) until analysis by Miami University CAWS laboratory (see below).

D. Plankton

Zooplankton were sampled using a Wisconsin-style plankton net with 48 μm mesh and 0.2 m opening diameter. Samples consisted of 2 vertical tows from 6 m depth to the surface. Two samples were collected from the lake center on each sampling date unless otherwise noted. Samples were collected in 125 mL bottles.

To sample phytoplankton, water was collected from 0.5 m, 2 m, 4 m, and 6 m using a 2.2 L Van Dorn-style collection bottle. Water from all depths were gently mixed in a bucket and two sub-samples of ~250 mL were collected from the mixed sample. The subsamples were screened with 153 μm mesh.

Zooplankton and phytoplankton samples were kept cool in the field and preserved with Lugol's iodine solution upon return to the lab.

APPENDIX II: Description of PLEON vendors

A. GreenWater Laboratories

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."

B. Miami University Center for Aquatic and Watershed Sciences

PLEON nutrient samples are analyzed by the Center for Aquatic and Watershed Sciences laboratory at Miami University.

C. Water Resources

PLEON plankton samples are enumerated by Ken Wagner of Water Resources.

Periphyton samples are concentrated by a factor of 5 before analysis. Concentrated samples are homogenized and subsamples are counted using a Palmer-Maloney counting chamber and phase-contrast microscopy (400x magnification). Biomass is determined using group-specific calculations.

Zooplankton samples are concentrated to at least 10,000x the original sample. Concentrated samples are homogenized and subsamples are counted using a Sedgewick-Rafter counting chamber and bright-field microscopy. Biomass is determined using group-specific calculations.

Appendix III: Literature Cited

1. Carlson, R. E. 1977. A trophic state index for lakes. Limnology and Oceanography 22(2): 361-369.
2. Swistock, B. 2015. Interpreting Water Tests for Ponds and Lakes. Retrieved on 22 February 2020, https://extension.psu.edu/interpreting-water-tests-for-ponds-and-lakes.
3. Williamson, C. E. et al. 2015. Ecological consequences of long-term browning in lakes. Scientific Reports 5:18666 DOI: 10.1038/srep18666

Appendix IV: Glossary

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Appendix V. Greenwater Laboratories Reports

Included as separate pdf files:

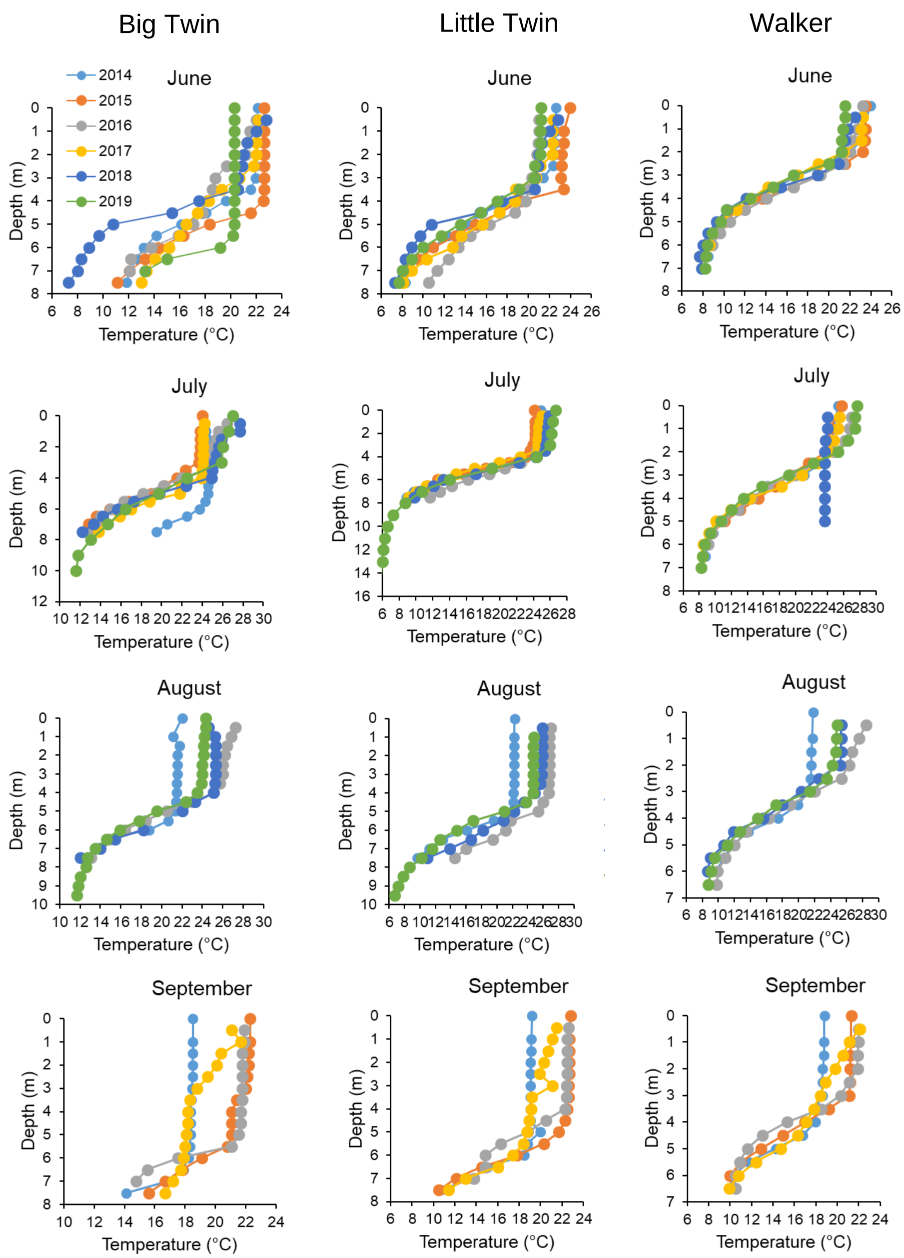
Big Twin Lake PTOX Cyanobacteria Screen 190717

Little Twin Lake PTOX Cyanobacteria Screen 190724

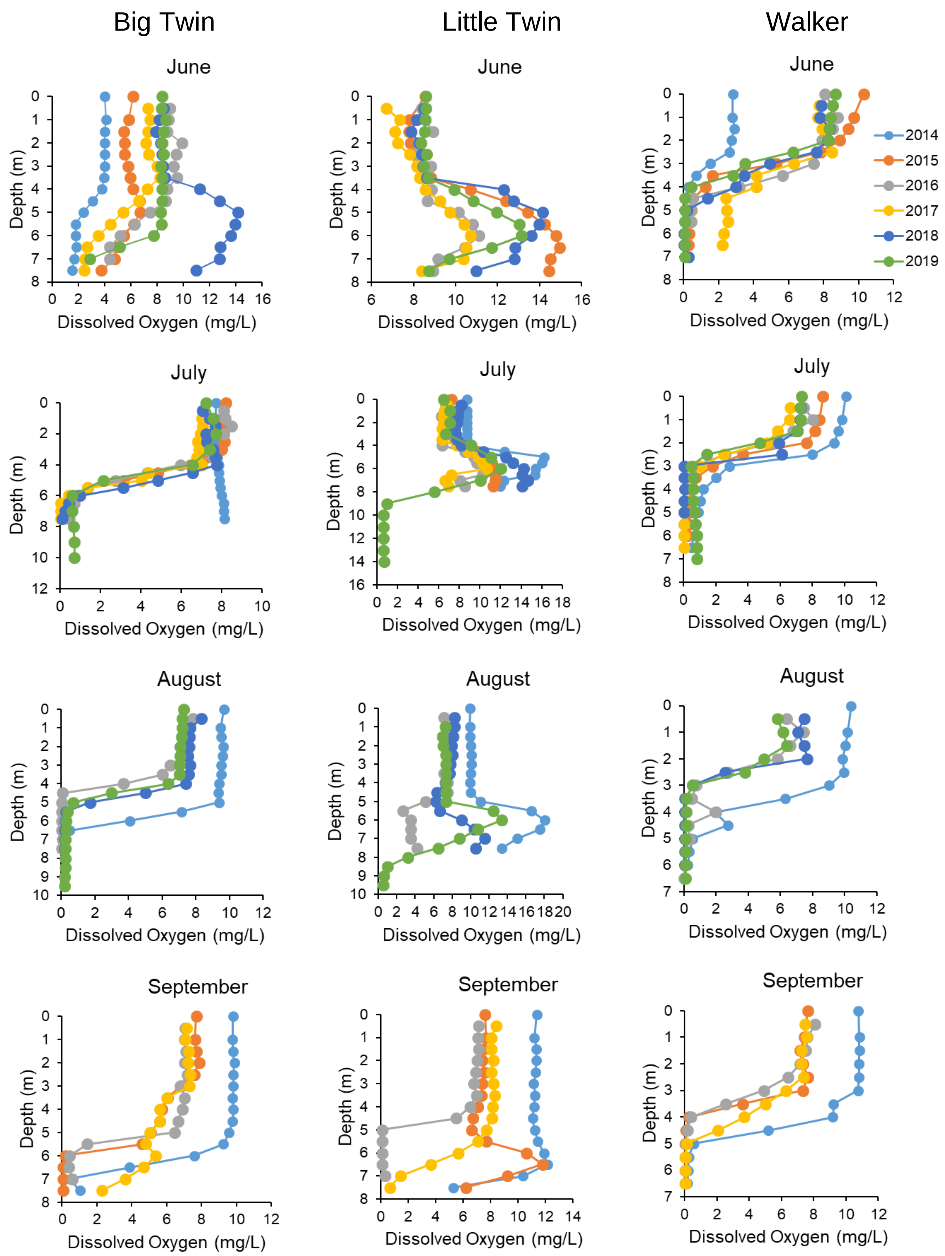
Walker PTOX Cyanobacteria Screen 190715

Appendix VI. Supplementary data

A. Monthly graphs of temperature since 2014



B. Monthly graphs of dissolved oxygen since 2014



C. Monthly surface conductivity and total dissolved solids since 2014

Table C1. Conductivity at 1 m.

	Big Twin				Little Twin				Walker			
	Jun	**Jul**	**Aug**	**Sep**	**Jun**	**Jul**	**Aug**	**Sep**	**Jun**	**Jul**	**Aug**	**Sep**
2014	60.0	ND	62.0	58.0	126.0	131.0	126.0	118.0	63.0	68.0	66.0	61.0
2015	234.0	ND	ND	74.1	462.0	ND	ND	142.6	267.0	ND	ND	79.7
2016	74.6	76.7	75.5	76.7	149.5	151.0	147.4	149.4	73.8	79.7	74.6	76.7
2017	70.3	71.1	ND	71.6	138.1	137.6	ND	136.5	90.2	70.2	ND	68.9
2018	139.2	75.0	46.2	ND	139.5	139.7	132.2	ND	70.0	70.0	63.9	ND
2019	63.0	65.4	80.1	ND	124.6	128.2	157.6	ND	55.8	62.6	80.1	ND

Table C2. Total dissolved solids (mg/L) at 1 m.

	Big Twin				Little Twin				Walker			
	Jun	**Jul**	**Aug**	**Sep**	**Jun**	**Jul**	**Aug**	**Sep**	**Jun**	**Jul**	**Aug**	**Sep**
2014	42	1	43	43	86	85	87	87	42	45	45	45
2015	159	ND	ND	ND	309	ND	ND	ND	179	ND	ND	ND
2016	49	50	49	50	98	98	96	97	48	52	49	50
2017	46	46	ND	47	90	90	ND	88	44	46	ND	45
2018	90	49	46	ND	90	91	86	ND	46	48	42	ND
2019	41	42	52	ND	81	83	103	ND	36	41	52	ND

Appendix VII. 2019 raw data

Attached as a separate excel file

2019_PLEON_TwinWalker_rawdata_toTWCWC