PLEON

POCONO LAKE ECOLOGICAL OBSERVATORY NETWORK

To the Twin and Walker Creeks Watershed Conservancy

Report of 2020 PLEON Sampling

From the Pocono Lakes Ecological Observatory Network

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I. Summary 2020: 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 2020. TWCWC monitoring included profiles of temperature, dissolved oxygen concentration, conductivity, pH, and TDS and samples for chlorophyll, total nitrogen concentration (TN), and total phosphorus concentration (TP). PLEON quantified dissolved organic carbon concentration (DOC) for the August collection.

PLEON also collected samples for the Pennsylvania Department of Environmental Protection and the PA Harmful Algae Task Force from all three lakes on 24 August 2020.

Table 1. Summary of PLEON 2020 monitoring activities by lake.

	Big Twin	**Little Twin**	**Walker**	PLEON Analysis	
TWCWC	20 June 20	20 June 20	21 June 20	Chlorophyll, TN, TP	
TWCWC	18 July 20	18 July 20	19 July 20	Chlorophyll, TN, TP	
PLEON	22 July 20	22 July 20	20 July 20	Depth profiles, Secchi depth, light profile, plankton communities (Twin lakes only)	Beth Norman
TWCWC	22 Aug 20	22 Aug 20	23 Aug 20	Chlorophyll, TN, TP, DOC	

B. Summary of water quality

Table 2. Summary of Big Twin Lake in 2020.

	20 June	18 July	22 July	22 August
Thermally stratified?	YES*	YES*	YES	YES*
Epilimnion depth (m)	4	5	3	6
Metalimnion depth (m)	>8	>8.5	9	>8
Secchi depth (m)	3.3	3	2.5	3.5
Vertical extinction coefficient (k)	—	—	1.01	—
$Z_{10\%}$ (m)	—	—	2.29	—
$Z_{1\%}$ (m)	—	—	4.58	—
DO at max depth (mg/L)	3.62**	1.73**	0.01	2.41
Epilimnetic chlorophyll concentration (µg/L)	1.88	1.23	—	2.35
TN in the epilimnion (mg/L)	0.53	0.43	—	0.50
TP in the epilimnion (µg/L)	$6.08^{\dagger}$	$3.54^{\dagger}$	—	$1.00^{\dagger}$
DOC in the epilimnion (mg/L)	—	—	—	3.75
TSI_{secchi}	42.8	44.2	46.8	41.9
$TSI_{chlorophyll}$	36.8	32.6	—	39.0
TSI_{TP}	29.8	22.1	—	$NC^{\dagger}$
Trophic classification* **	OLIGOTROPHIC	OLIGOTROPHIC	—	OLIGOTROPHIC
Zooplankton density (#/L)	—	—	540	—
Most abundant zooplankton taxa	—	—	ROTIFERA	—
Phytoplankton density (cells/mL)	—	—	1,985	—
Most abundant phytoplankton	—	—	CYANOPHYTA	—
PTOX cyanobacteria found?§	—	—	—	YES
Toxin testing recommended?§	—	—	—	YES
Toxin concentration§	—	—	—	BELOW DETECTION

**incomplete profile, **at deepest measured, not maximum depth, †below analytical detection ***According to $TSI_{chlorophyll}$. Classification depends on TSI metric. §part of PA DEP/Harmful Algae Task Force survey, not PLEON, from 24 Aug 2020 samples.*

Table 3. Summary of Little Twin Lake in 2020.

	20 June	18 July	22 July	22 August
Thermally stratified?	YES	YES*	YES	YES*
Epilimnion depth (m)	4	5	3	6
Metalimnion depth (m)	7.5	>9	11	>8
Secchi depth (m)	3	3.5	4.5	3
Vertical extinction coefficient (k)	—	—	0.41	—
$Z_{10\%}$ (m)	—	—	5.60	—
$Z_{1\%}$ (m)	—	—	11.2	—
DO at max depth (mg/L)	8.08**	4.07**	0.01	6.83**
Epilimnetic chlorophyll concentration (µg/L)	1.55	0.16	—	2.12
TN in the epilimnion (mg/L)	0.99	0.94	—	0.90
TP in the epilimnion (µg/L)	18.7	18.7	—	†
DOC in the epilimnion (mg/L)				2.87
TSI_{secchi}	44.2	41.9	38.3	44.2
$TSI_{chlorophyll}$	34.9	12.4	—	38.0
TSI_{TP}	45.7	45.7	—	NC†
Trophic classification* **	OLIGOTROPHIC	OLIGOTROPHIC	—	OLIGOTROPHIC
Zooplankton density (#/L)	—	—	341	—
Most abundant zooplankton taxa	—	—	ROTIFERA	—
Phytoplankton density (cells/mL)	—	—	2,486	—
Most abundant phytoplankton	—	—	CYANOPHYTA	—
PTOX cyanobacteria found?§	—	—	—	YES
Toxin testing recommended?§	—	—	—	YES
Toxin concentration§	—	—	—	BELOW DETECTION

**incomplete profile, **at deepest measured, not maximum depth, ***According to $TSI_{chlorophyll}$. Classification depends on TSI metric. †below the analytical detection limit. §part of PA DEP/Harmful Algae Task Force survey, not PLEON, from 24 Aug 2020 samples.*

Table 4. Summary of Walker Lake in 2020.

	21 June	19 July	20 July	23 August
Thermally stratified?	YES	PARTIAL	YES	PARTIAL*
Epilimnion depth (m)	3	3	2	2
Metalimnion depth (m)	6	6.5[†]	7	>6.5
Secchi depth (m)	2	1.2	1.5	1.5
Vertical extinction coefficient (k)	—	—	1.15	—
$Z_{10\%}$ (m)	—	—	2.00	—
$Z_{1\%}$ (m)	—	—	4.00	—
DO at max depth (mg/L)	0.17	0.17	0.02	0.13
Epilimnetic chlorophyll concentration (µg/L)	3.94	0.920	—	9.81
TN in the epilimnion (mg/L)	0.83	0.66	—	0.85
TP in the epilimnion (µg/L)	11.2	6.08[€]	—	16.2
DOC in the epilimnion (mg/L)	—	—	—	5.11
TSI_{secchi}	50.0	57.4	54.2	54.2
$TSI_{chlorophyll}$	44.0	29.8	—	53.0
TSI_{TP}	38.4	29.8	—	43.7
Trophic classification* **	MESOTROPHIC	OLIGOTROPHIC	—	MESOTROPHIC
PTOX cyanobacteria found?[§]	—	—	—	YES
Toxin testing recommended?[§]	—	—	—	YES
Toxin concentration[§]	—	—	—	BELOW DETECTION

**incomplete profile, **at deepest measured, not maximum depth, ***According to $TSI_{chlorophyll}$. Classification depends on TSI metric. [†]maximum depth. [€]below the analytical detection limit. [§]part of PA DEP/Harmful Algae Task Force survey, not PLEON, from 24 Aug 2020 samples.*

II. 2020 Physical Profile Results

A. Temperature and Secchi Depth

The 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. The July PLEON sampling offers complete profiles of all three lakes. At this time, average epilimnetic temperatures of the three lakes were similar (~28°C). Average deep water temperature was warmest in Big Twin (10.4°C), followed by Walker (9.1°C) and Little Twin (7.7°C).

Secchi depth ranged from 2.5-3.5 m in Big Twin, from 3-4.5 m in Little Twin, and from 1.2-1.5 m in Walker (Figure 2). 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 status (Table 5). Average TSI_{Secchi} of Big Twin, Little Twin, and Walker in 2020 was 43.9, 42.2 and 53.9, respectively. Big and Little Twin are classified as mesotrophic while Walker is classified as eutrophic according to this metric.

Table 5. Trophic classification description

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

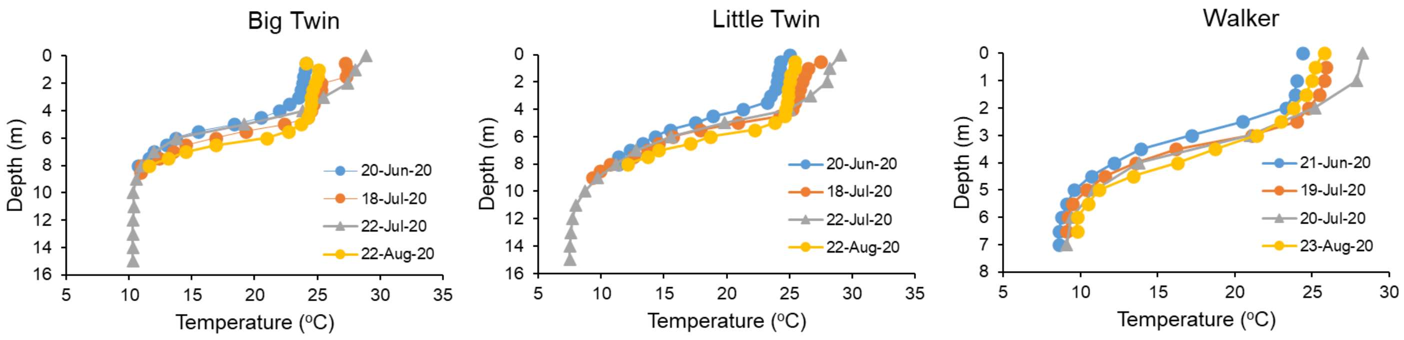

Figure 1. Temperature profiles of Twin and Walker lakes during 2020. Note differences in scale.

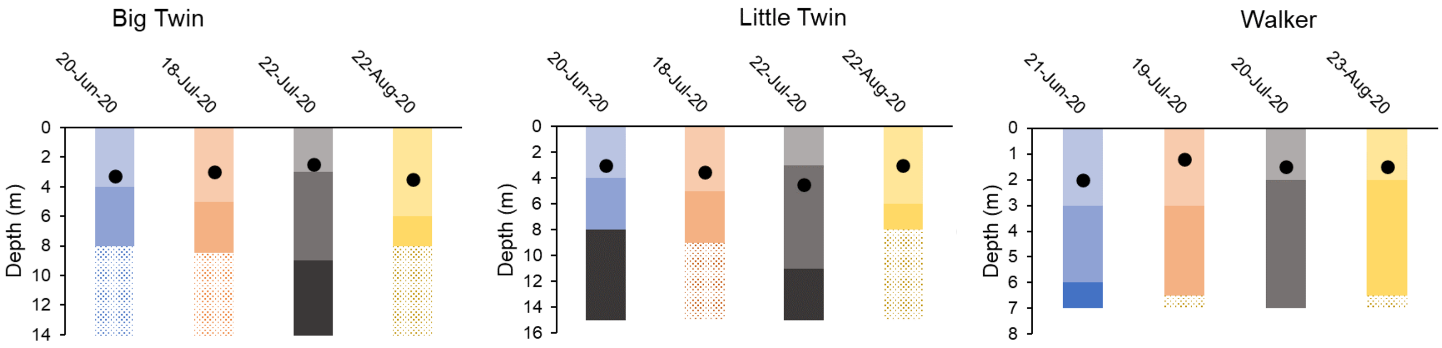

Figure 2. Stratification of Twin and Walker lakes during 2020. Note differences in scale. Bar shading denotes stratification. Closed circles show Secchi depth. Depths greater than cable length are hashed. Note difference in scale among panels.

B. Dissolved Oxygen

Dissolved oxygen concentration (DO) profiles were variable within lakes in 2020 with some problematic data (Figure 3).

DO in Big and Little Twin was consistently below 2 mg/L in the epilimion on 18 July. While it is common for deep water to be hypoxic, surface waters are usually well oxygenated. In fact, with the exception of readings from Big Twin in June, all of the DO readings taken by TWCWC are at the low end or lower than those recorded by PLEON (20-22 July) and by TWCWC in previous years (Appendix VI). This suggests that there was an issue with the TWCWC DO meter. Due to this possibility, the comparisons below are made using the data collected by PLEON in July.

DO fell below the 2 mg/L hypoxia threshold at 5 m in Big Twin, 9 m in Little Twin, and 4 m in Walker. Little Twin had a metalimnetic DO maximum that peaked at 6 m suggesting high algal abundance in the middle waters.

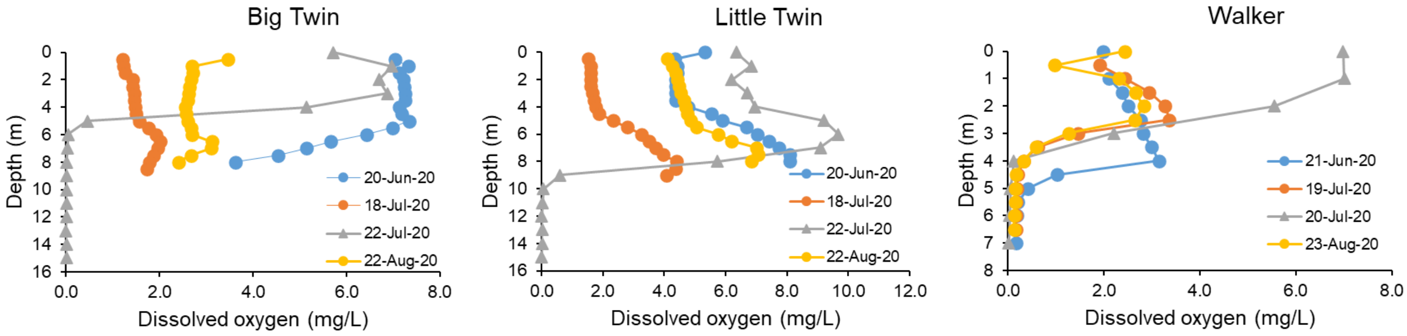

Figure 3. Dissolved oxygen profiles of Twin and Walker lakes in 2020. Note differences in scale.

C. Conductivity

Conductivity was highest in Little Twin (summer average at 1 m = 157 µS/cm), followed by Big Twin (82 µS/cm) and Walker (79 µS/cm; Figure 4). Epilimnetic conductivity was similar across months within lakes. Conductivity was generally greater in the deep waters compared to the surface, with the exception of the PLEON July sampling of Little Twin which showed higher conductivity at the surface.

There were 2 very low, outlying readings in the 2020 data: one in August in Big Twin and one in June in Little Twin. These data were likely read or recorded incorrectly or the probe may not have been properly submerged.

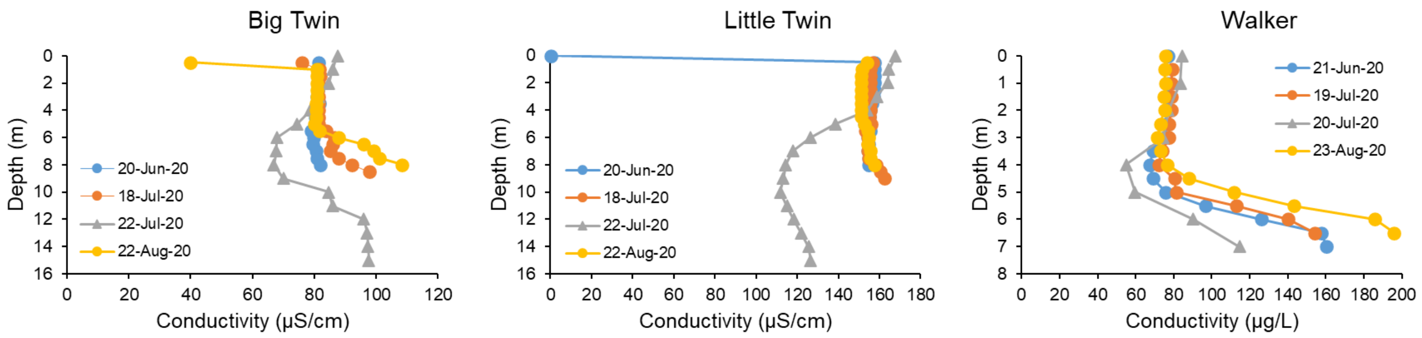

Figure 4. Conductivity profiles of Twin and Walker lakes during 2020. Note differences in scale.

D. pH

pH ranged from 6.3-8.6 in Big Twin and 6.7-8.2 in Little Twin. pH was more variable in Walker, ranging from 5.4-8.2 in Walker (Figure 5). pH generally declined with depth, although pH began to increase near the sediments in Walker and Big Twin (only one complete profile).

There were 2 outlying readings in the 2020 data: one in August in Big Twin and one in June in Little Twin. These data were likely read or recorded incorrectly or the probe may not have been properly submerged.

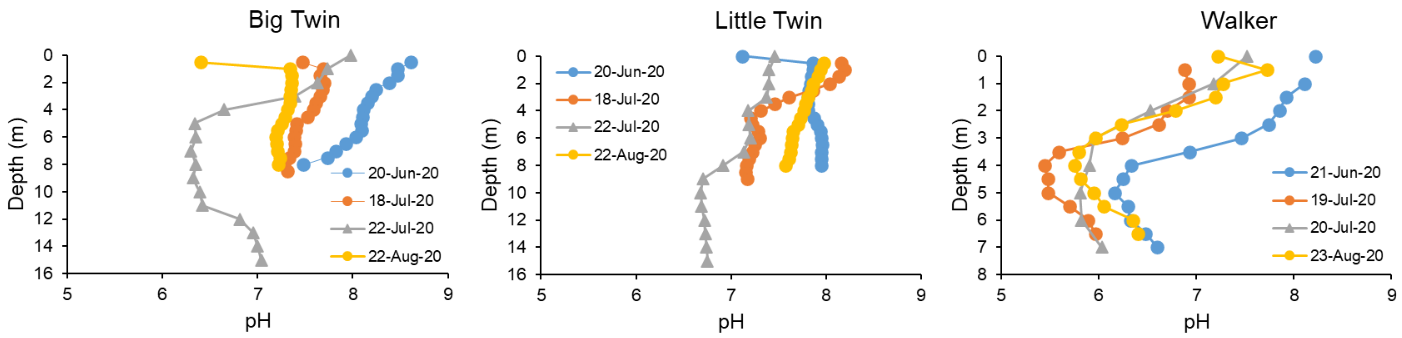

Figure 5. pH profiles of Twin and Walker lakes during 2020. Note differences in scale.

E. Total Dissolved Solids

Total dissolved solids concentration (TDS) was measured in the first 7-9 m in all three lakes, capturing a complete profile in Walker but not in Big or Little Twin (Figure 6). TDS was highest in Little Twin for the first 5 m (ranging ~98-105 mg/L) compared to the other lakes. TDS increased with depth below 5 m in Big Twin and Walker in all seasons, reaching a maximum of 70 mg/L in Big Twin (22 Aug) and 127 mg/L in Walker (23 Aug).

There were 2 very low, outlying readings in the 2020 data: one in August in Big Twin and one in June in Little Twin. These data were likely read or recorded incorrectly or the probe may not have been properly submerged.

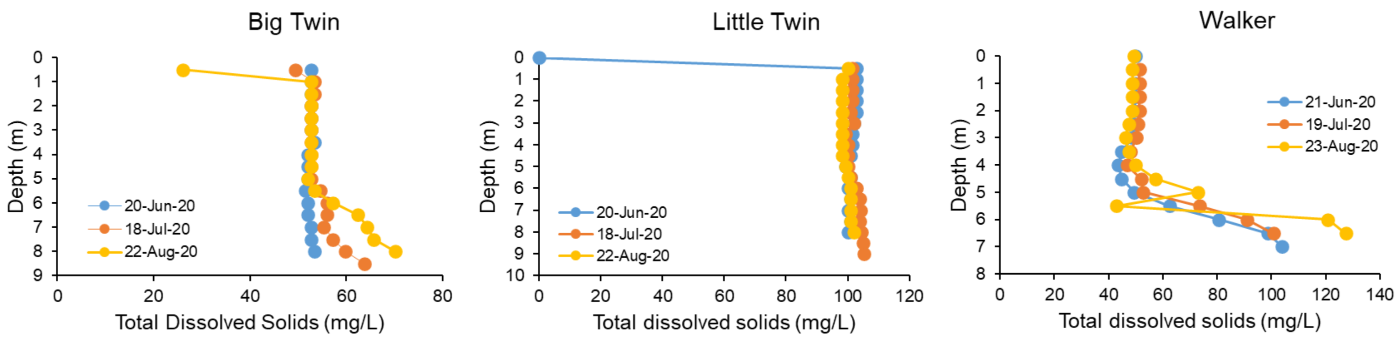

Figure 6. Total dissolved solids profiles of Twin and Walker lakes during 2020. Note scale differences.

F. Light

Dissolved and particulate material affect the rate at which light intensity attenuates with depth. Light intensity declines exponentially with depth (Figure 7) 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 (smallest *k*) followed by Big Twin and Walker (Table 6).

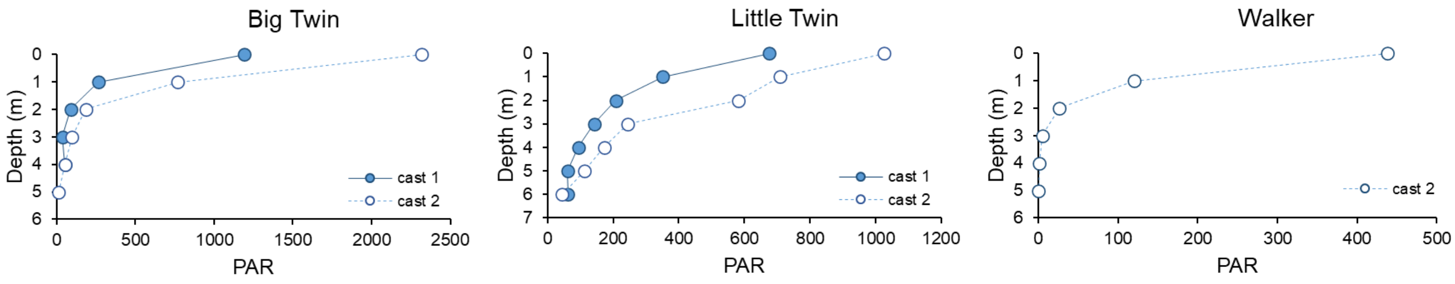

Figure 7. Depth profiles of PAR in Twin and Walker lakes in July 2020. Note differences in scale. Cast 1 in Walker was discarded due to lack of data points.

Table 6. Light intensity parameters calculated from PAR profiles in Twin and Walker lakes in July 2020. Parameters were calculated from the Cast 2 in Big Twin and Walker and Cast 1 in Little Twin.

	k	$Z_{10\%}$	$Z_{1\%}$
Big Twin	1.01	2.29	4.58
Little Twin	0.41	5.60	11.2
Walker	1.43	1.61	3.22

III. Chlorophyll Results

Chlorophyll *a* concentration (chla) at 0.5 m was lowest during July in all the lakes (Figure 8). Surface chla was similar in June and August in Big Twin and Little Twin. Walker had more chla in surface samples in August. Little Twin had the lowest chla of the three lakes and Walker had the highest, except for in July when Walker chla was similar to that measured in the Twin lakes.

Composite samples generally had more chla than the surface samples. This difference was greater than 50% in July in Little Twin and in June in Walker. In the case of Little Twin, algae may have been concentrated at middle depths to avoid exposure to harmful UV radiation. This is supported by the metalimnetic oxygen maximum observed in this lake. UV avoidance may also explain the algae distribution in Walker although Walker is not as transparent as Little Twin.

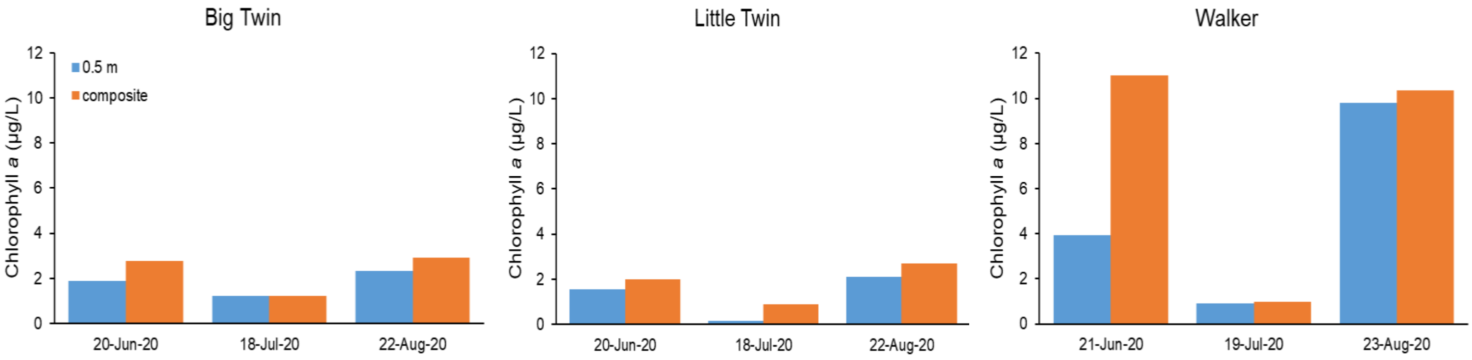

Figure 8. Chlorophyll* a *concentration in Twin and Walker lakes in 2020. Bars are single samples.

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

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

The average $\text{TSI}_{\text{chlorophyll}}$ of Big Twin, Little Twin, and Walker was 36.1, 28.4, and 42.3, 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 and total phosphorus

Total nitrogen concentration (TN) in the surface waters of Big Twin, Little Twin, and Walker averaged 0.49 mg/L, 0.94 mg/L, and 0.78 mg/L, respectively, over the summer (Figure 9). TN of composite samples were generally lower in all lakes, indicating less nitrogen in deep waters. TN concentration in all three lakes were below the Penn State Extension office suggested 3 mg/L threshold for nutrient pollution[2].

Total phosphorus concentration (TP) in the surface waters of Big Twin, Little Twin, and Walker averaged 3.54 µg/L, 15.8 µg/L, and 11.15 µg/L, respectively, over the summer (Figure 9). Composite samples in Big Twin and Little Twin were more concentrated than the surface during the August sampling, indicating more TP in deep waters. In contrast, the Walker composite sample had 2x the TP compared to the surface samples in June. TP concentration in all three lakes was generally below the Penn State Extension office suggested 25 µg/L threshold for nutrient pollution[2].

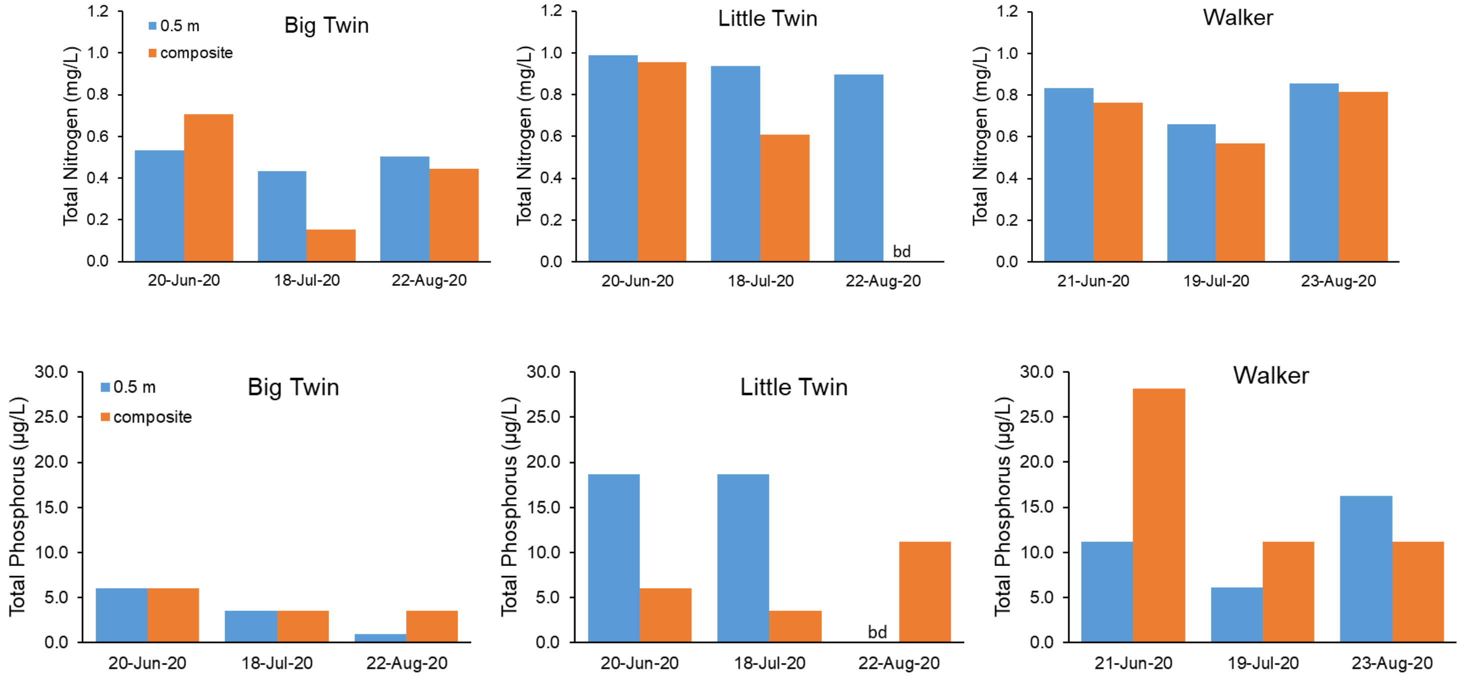

Figure 9. TN and TP in Twin and Walker lakes in 2020. Bars are single samples. The TP analytical detection limit is 10 µg/L.

TSI can be calculated from TP 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 22.1, 41.8, and 38.4, respectively. Big Twin and Walker are classified as oligotrophic and Little Twin as mesotrophic according to this metric (Table 5).

C. Dissolved organic carbon

Dissolved organic carbon concentration (DOC) was measured in the August 2020 samples, with the exception of the Walker composite sample. DOC was greatest in Walker, followed by Big Twin, with Little Twin having the least DOC (Figure 10). Composite samples from the Twin lakes had slightly more DOC compared to the surface samples.

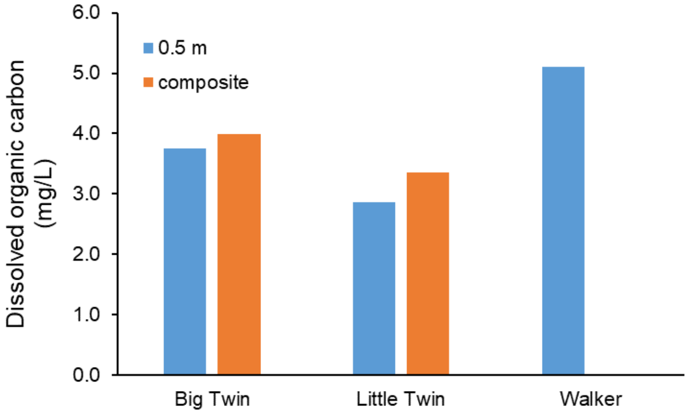

Figure 10. DOC in Twin and Walker lakes in August 2020 samples. Bars are single samples. Walker composite sample was not analyzed.

V. Plankton Communities

A. Zooplankton

Twin Lakes zooplankton were numerically dominated by rotifers, followed by copepods and protozoans in Big Twin and copepods and cladocerans in Little Twin (Table 7). Copepods dominated Big Twin zooplankton by mass, followed by rotifers and cladocerans while cladocerans dominated Little Twin zooplankton by mass, followed by copepods and other zooplankton.

Biological communities can be described using richness and diversity. Richness is the number of taxa present while diversity accounts for both the number of taxa and the distribution of individuals among taxa. Average zooplankton richness in Big Twin and Little Twin was 11.5 and 16, respectively and average diversity (Shannon-Wiener Index) was 0.84 and 0.95, respectively.

Table 7. Zooplankton density and biomass (averages 2 replicates) of Twin lakes on 22 July 2020.

	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	**36**	**7**	**4**	**1**	**17**	**5**	**<1**	**<1**
Ciliophora	36	7	4	1	17	5	<1	<1
ROTIFERA	**403**	**75**	**28**	**11**	**188**	**55**	**15**	**3**
Conochilus	126	23	5	2	22	7	1	0
Kellicottia	5	1	<1	<1	16	5	1	0
Keratella	144	27	13	5	107	31	10	2
Polyarthra	97	18	9	3	40	12	4	1
Trichocerca	31	6	1	<1	3	1	<1	<1
COPEPODA	**91**	**17**	**203**	**80**	**79**	**23**	**198**	**44**
Copepoda-Cyclopoida	37	7	62	24	21	6	39	9
Cyclops group	13	2	31	12	10	3	25	6
Mesocyclops	25	5	31	12	11	3	14	3
Copepoda-Calanoida	1	<1	<1	<1	3	1	1	<1
Diaptomus group	1	<1	<1	<1	3	1	1	<1
Other Copepoda-Nauplii	53	10	140	55	54	16	158	35
CLADOCERA	**10**	**2**	**20**	**8**	**57**	**17**	**217**	**49**
Bosmina	5	1	7	3	5	1	4	1
Ceriodaphnia	5	1	13	5	27	8	114	26
Daphnia ambigua	–	–	–	–	17	5	64	14
Diaphanosoma	–	–	–	–	5	1	9	2
Holopedium	–	–	–	–	3	1	26	6
OTHER ZOOPLANKTON	–	–	–	–	**<1**	**<1**	**16**	**4**
Chaoboridae	–	–	–	–	<1	<1	16	4
TOTAL	**540**		**254**		**341**		**446**	

B. Phytoplankton

Cyanophyta, or cyanobacteria, were the numerically dominant algal group in both Big Twin and Little Twin (34% and 59% of the communities, respectively), followed by Chlorophyta (green algae) and Bacillariophyta (diatoms; Table 8). Total abundance was higher in Little Twin compared to Big Twin, driven by the comparatively larger number of cyanobacteria. Phytoplankton biomass in both lakes was spread more evenly among groups with Chrysophyta (golden-brown algae) dominant by mass.

Average phytoplankton richness was 17 in Big Twin and Little Twin and average diversity (measured using the Shannon-Wiener Index) in the two lakes was 1.06 and 0.94, respectively.

Table 8. Phytoplankton communities from Twin lakes on 22 July 2020. 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	**283**	**14**	**227**	**20**	**210**	**8**	**155**	**11**
Centric Diatoms	—	—	—	—	12	<1	14	1
Araphid Pennate Diatoms	283	14	227	20	198	8	141	10
CHLOROPHYTA	**566**	**29**	**187**	**17**	**466**	**19**	**154**	**11**
Flagellated Chlorophytes	—	—	—	—	180	7	36	3
Coccoid/Colonial Chlorophytes	307	15	78	7	97	4	17	1
Filamentous Chlorophytes	218	11	44	4	154	6	31	2
Desmids	41	2	66	6	35	1	71	5
CHRYSOPHYTA	**228**	**11**	**407**	**37**	**256**	**10**	**313**	**22**
Flagellated Classic Chrysophytes	228	11	407	37	256	10	313	22
CRYPTOPHYTA	**173**	**9**	**35**	**3**	**77**	**3**	**233**	**16**
CYANOPHYTA	**668**	**34**	**76**	**7**	**1,465**	**59**	**37**	**3**
Unicellular and Colonial Forms	306	15	3	<1	—	—	—	—
Filamentous N Fixers	362	18	72	6.5	115	5	23	2
Filamentous Non-N Fixers	—	—	—	—	1,350	54	14	1
EUGLENOPHYTA	**60**	**3**	**60**	**5**	—	—	—	—
PYRRHOPHYTA	**7**	**<1**	**120**	**11**	**12**	**<1**	**538**	**38**
TOTAL	**1,985**		**1,111**		**2,486**		**1,430**	

Both lakes contained cyanobacteria that can produce cyanotoxins, including *Woronichinia*, *Dolichospermum*, *Oscillatoria*, and *Pseudanabaena* (Figure 11). 100% and 35% of the cyanobacteria were potentially toxigenic in Big and Little Twin, respectively

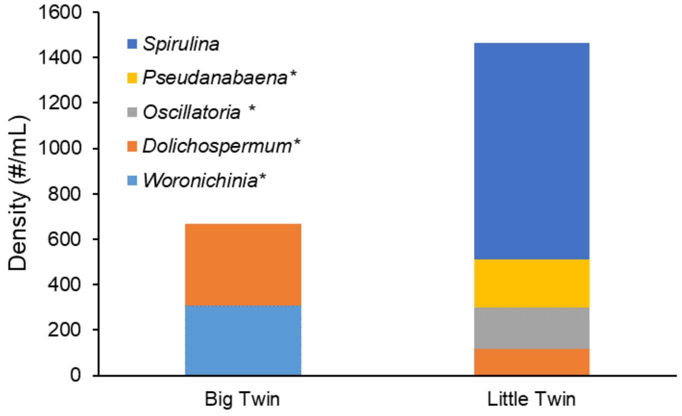

Figure 11. Cyanobacteria density (averages of 2 replicates) in Big and Little Twin on 22 July 2020. * potentially toxigenic.

VI. PA Harmful Algal Bloom Task Force Cyanobacteria Screens

On 24 August 2020, surface samples were collected off docks in each lake. Samples were screened by Greenwater Laboratories and the Pennsylvania Bureau of Laboratories for potentially toxigenic (PTOX) cyanobacteria and tested for cyanotoxin concentration (Appendix V). This screening was not a part of the PLEON package. For more information regarding these samples, contact HABS@pa.gov.

PTOX genera were found in all three lakes and the concentrations of 3-4 cyanotoxins were quantified (Table 9). The concentration of all toxins analyzed was below detection.

Table 9. Summary of PA Harmful Algae Bloom Task Force data from Twin and Walker lakes.

	Observed Genera	Count*	Toxins analyzed**	Toxin results**
Big Twin	*Dolichospermum*	>120	MIC, SAX, ANA, CYL	All <MDL
Little Twin	*Dolichospermum* *Aphanizomenon/Chrysosporum*	>10 >2	MIC, SAX, ANA, CYL	All <MDL
Walker	*Chrysosporum*	>130	CYL, SAX, ANA	All <MDL

**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. MIC and SAX quantified using enzyme-linked immunosorbent assays (ELISA), CYL and ANA quantified using liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS). MDL = minimum detection limit (0.3 ng/mL for MIC, all others 0.05 ng/mL).*

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 2014-2018 were provided by the TWCWC in the form of yearly “state of the lake” reports by FX Browne.

B. Temperature, dissolved oxygen, and water clarity

July surface (1 m depth) temperatures have been getting warmer in Big Twin and Little Twin (Figure 12). This trend is statistically significant (linear regression) in both lakes. Surface temperatures in Walker have been more variable and although surface waters appear to be warming over time, the trend is not statistically significant.

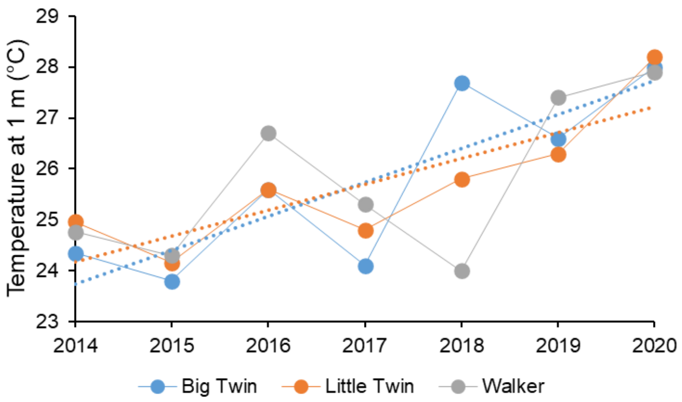

Figure 12. Surface temperatures (1 m) measured in July in Twin and Walker lakes since 2014. Dotted lines are linear regressions of trends in Big Twin (blue; y=0.6661x-1317.7; r^2=0.62; p=*0.02) and Little Twin (orange; y=0.5071x-997.22;* r^2*=0.63;* p=*0.02).*

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.

Since 2014, Walker has consistently developed a hypoxic/anoxic hypolimnion in July, the month with the most complete dataset (Figure 13). The depth of oxygen depletion (defined as <2.0 mg/L) has ranged from 4-2.5 m since 2014. Big Twin and Little Twin also have hypoxic hypolimnions but only 2 years of data are available. In those 2 years, the depth of oxygen depletion in Big Twin has been between 5-6 m while this depth has been 9 m in Little Twin. Both Twin lakes are ~15 m deep.

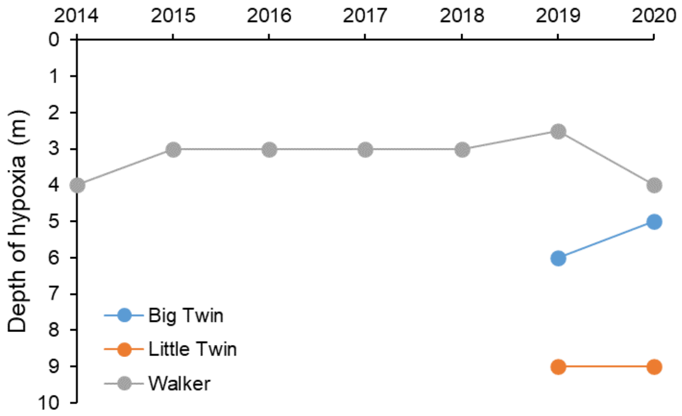

Figure 13. The depth of hypoxia/anoxia (<2 mg DO/L) in Twin and Walker lakes over time. 2019 ans 2020 data are from July PLEON sampling.

Little Twin has been the most transparent of the three lakes since 2014, followed by Big Twin and Walker (Figure 14). Secchi depth has remained fairly consistent in all three lakes since 2014.

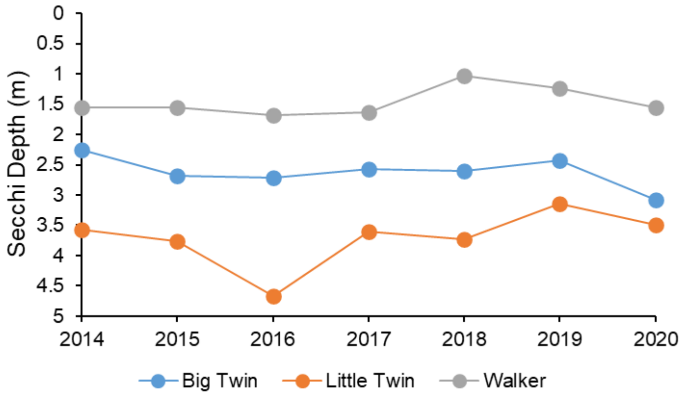

Figure 14. Average Secchi depth (left) and TSI_{Secchi} (right) in Twin and Walker lakes since 2014. Horizontal dashed lines in right panel show boundaries of oligotrophic (<40), mesotrophic (40-50), eutrophic (50-70), and hypereutrophic (>70) classifications.

C. Chlorophyll and phosphorus

Patterns of chlorophyll *a*, a proxy for algal biomass, have generally been similar over time in all three lakes (Figure 15). 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 has been declining since 2017.

Prior to 2020, the temporal pattern of TP was similar among all lakes with a peak in 2016 (Figure 15). TP has generally been least in Little Twin and greatest in Walker since 2014. Since 2017, TP in Big Twin and Little Twin have converged while TP in Walker has been roughly 2x that of the other lakes. 2020 is a departure from this trend with TP in Little Twin greater than Walker, due both to an increase in TP in Little Twin and a decrease in Walker.

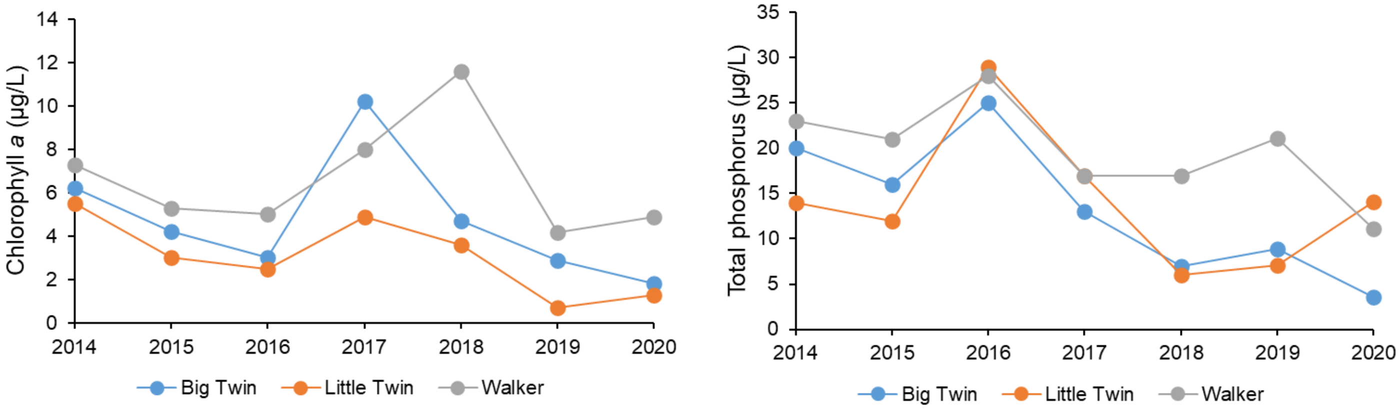

Figure 15. Average chlorophyll** a **and TP concentration in Twin and Walker lakes since 2014.

PLEON used different nutrient analysis technique in 2020. Preliminary comparisons with the previous technique suggests that the new procedure over estimates TN and slightly underestimates TP. This report may be amended depending on a more complete comparative analysis.

D. Trophic status

The trophic status of Twin and Walker lakes has been fairly consistent since 2014, with variation depending on the metric used (Figure 16). Big Twin has generally been mesotrophic, trending toward oligotrophy since 2017. Little Twin has generally been oligo-mesotrophic and is showing signs of trending toward mesotrophy in the past 3 years. Walker has generally been meso-eutrophic.

Since 2014, TSI_{TP} has generally been lower in Twin and Walker lakes compared to $\text{TSI}_{\text{chlorophyll}}$ while $\text{TSI}_{\text{Secchi}}$ generally been higher. Trophic status is defined by algae production. Therefore, chlorophyll concentration is the most direct way to assess trophic status of the three metrics.

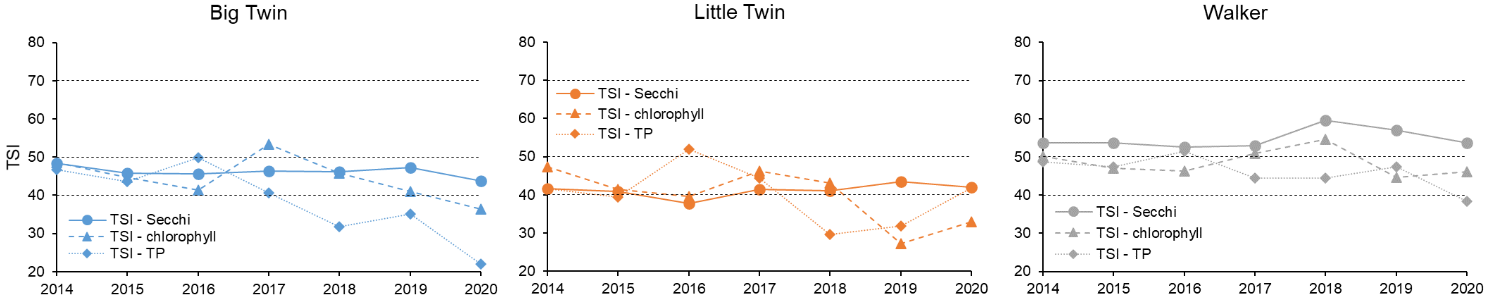

Figure 16. TSI of Twin and Walker lakes since 2014. Horizontal dashed lines show boundaries of oligotrophic (<40), mesotrophic (40-50), eutrophic (50-70), and hypereutrophic (>70) classifications.

E. Plankton Communities

PLEON has quantified plankton abundance and biomass in Big Twin and Little Twin since 2019.

Zooplankton density was greater in 2020 compared to 2019 in both lakes, by almost 5x in Big Twin and by almost 3x in Little Twin (Figure 17). Zooplankton community composition was generally similar within lakes across years. Rotifers were the dominant group in 2019 and 2020 in both lakes. The relative abundance of protozoans increased in 2020 in both lakes and cladoceran relative abundance increased in 2020 in Little Twin.

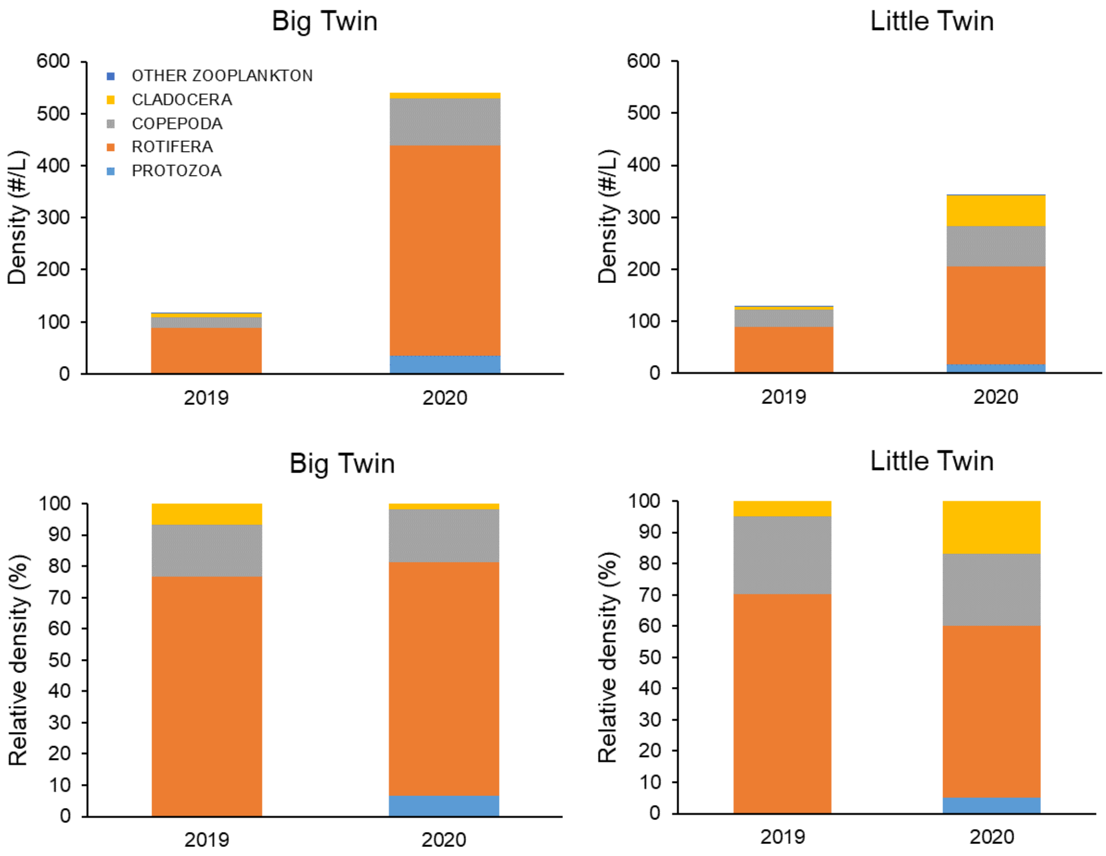

Figure 17. Zooplankton community density and relative density in the Twin lakes since 2019.

The zooplankton community in Big Twin had similar richness and mean length across years with a slight increase in diversity (suggesting a more even distribution of individuals among the same number of taxa in 2020; Table 10). The average length of individuals is an important metric for zooplankton communities as large zooplankton are efficient “algae eaters” and can regulate algae communities. Richness, diversity, and average length were greater in the 2020 Little Twin zooplankton communities compared to 2019. However, two years of data is not enough to identify ecologically significant trends.

Table 10. Zooplankton community metrics in Big Twin and Little Twin since 2019.

		Richness	Diversity*	Mean length (mm)
Big Twin	**2019**	12	0.72	0.17
	2020	11.5	0.84	0.16
Little Twin	**2019**	13.5	0.72	0.19
	2020	16	0.95	0.27

**Shannon-Wiener diversity index*

In Big Twin, phytoplankton density decreased in 2020 by almost 50% compared to 2019 (Figure 18). This was due to a decrease in Cyanophyta (by ~75%) and to a lesser extent, Chlorophyta. Bacillariophyta, Chrysophyta, and Euglenophyta made up larger proportions of phytoplankton density in 2020 than in 2019. In Little Twin, phytoplankton density showed the opposite pattern, increasing slightly in 2020 compared to 2019 (Figure 18). This was due to an increase in Chlorophyta. Chlorophyta made up a larger proportion of the phytoplankton community in 2020 compared to 2019 while the relative abundance of Chrysophyta decreased.

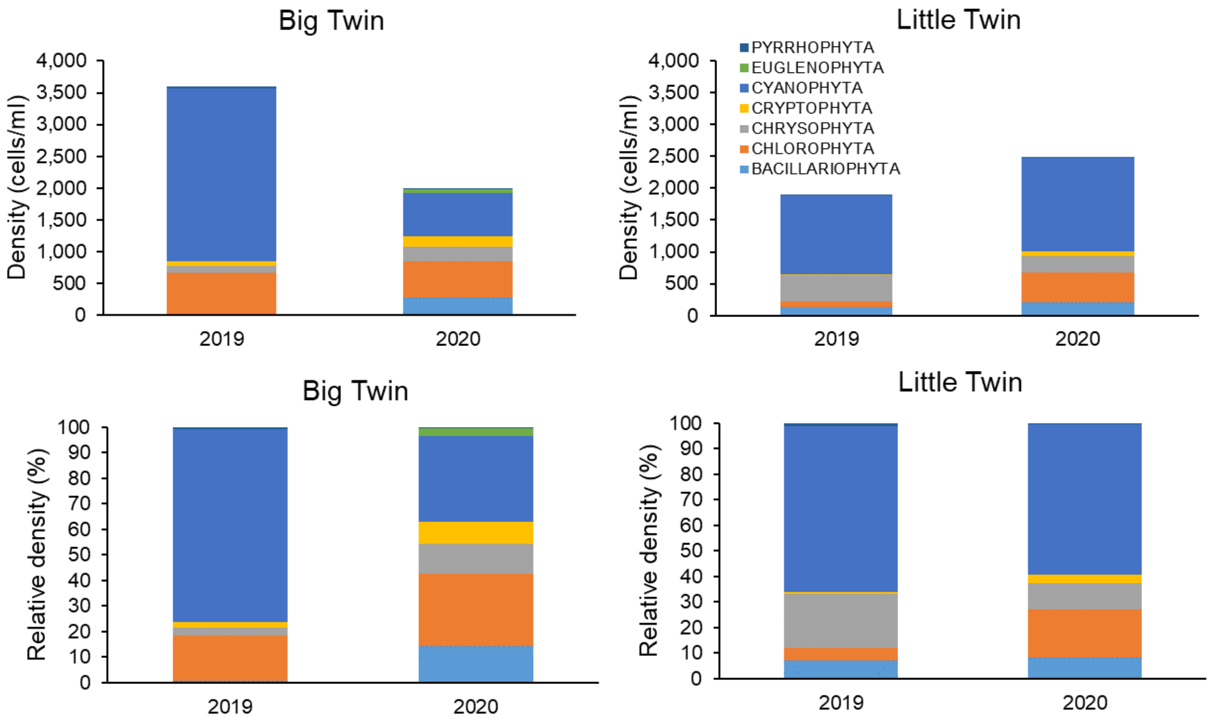

Figure 18. Phytoplankton community density and relative density in the Twin lakes since 2019.

Potentially toxigenic (PTOX) cyanobacteria genera were present in both years (Figure 19). In Big Twin, PTOX genera made up 100% of the cyanobacteria community in 2019 and 2020 but the genera differed among years with *Dolichospermum* less abundant in 2020 and *Woronichinia* more so. Overall PTOX density was lower in 2020 compared to 2019 in this lake.

In Little Twin, the density of cyanobacteria increased in 2020 compared to 2019 but >50% of the community was *Spirulina*, a genera not known to produce toxins. This was a shift from 2019 when 100% of the cyanobacteria was the PTOX genera *Dolichospermum*. Approximately 30% of the cyanobacteria found in Little Twin in 2020 belonged to PTOX genera.

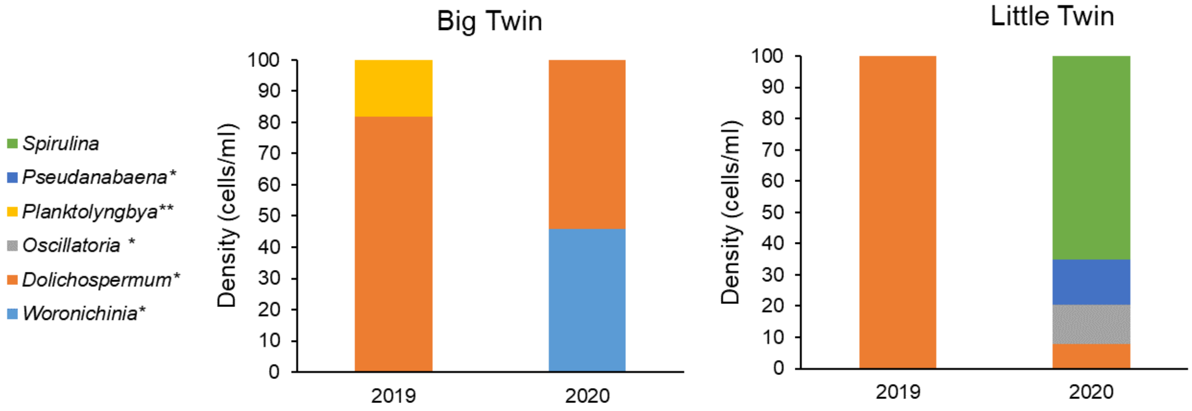

Figure 19. Relative density of genera within the cyanobacteria community the Twin lakes since 2019. *genera known to produce cyanotoxins **genera thought to produce cyanotoxins.

Phytoplankton taxonomic richness ranged from 17-20 and from 12-17 since 2019 in Big and Little Twin, respectively (Table 11). Phytoplankton diversity ranged from 0.64-1.06 and from 0.59-0.94 in Big and Little Twin, respectively. Temporal trends are not identifiable from 2 years; more monitoring is needed to determine changes over time.

Table 11. Phytoplankton community metrics in Big Twin and Little Twin since 2019.

		Richness	Diversity*
Big Twin	**2019**	20	0.64
	2020	17	1.06
Little Twin	**2019**	12	0.59
	2020	17	0.94

**Shannon-Wiener diversity index*

F. PTOX cyanobacteria

Samples from all three lakes were screened for potentially toxigenic (PTOX) cyanobacteria in 2019 and 2020, as part of the Pocono Lakes HABs Survey (Lauren Knose, Miami University), and by the PA Harmful Algae Bloom Task Force monitoring program, respectively. Both initiatives used Greenwater Laboratories for screening and toxin analysis (2020 only). Results are shown in Table 12.

Table 12. Results of PTOX screens of Twin and Walker lakes.

		PTOX genera	Toxin testing recommended?*	Toxin results***
Big Twin	17 Jul 2019	*Dolichospermum*	NO**	—
	24 Aug 2020	*Dolichospermum*	YES	<MDL
Little Twin	24 July2019	*Dolichospermum*	NO**	—
	24 Aug 2020	*Dolichospermum* *Aphanizomenon/Chrysosporum*	YES	<MDL
Walker	15 Jul 2019	*Aphanizomenon*	NO**	—
	24 Aug 2020	*Chrysosporum*	YES	<MDL

**by Greenwater Laboratory **due to low abundance, ***MDL=minimum detection limit*

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

A. Description of PLEON Lakes

The PLEON dataset consists of 19 lakes in Pike, Wayne, and Monroe Counties, ranging from ~80,000-1,130,000 m^2 (mean of 390,000 m^2) in surface area, ~1,400-7,800 m (mean of 3,280 m) in shoreline and ~2-23 m (mean of 12 m) in depth (Figure 20).

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

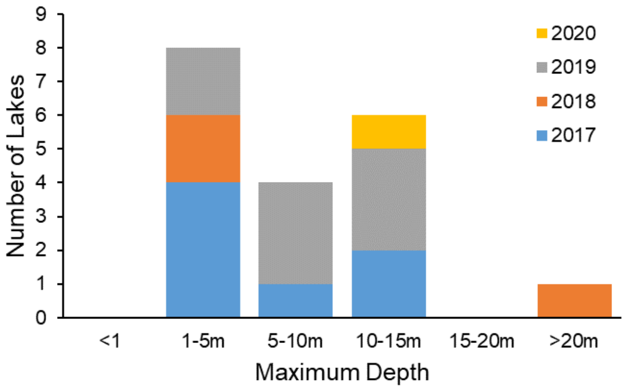

Figure 20. Distribution of PLEON lakes by maximum depth (m) and year PLEON monitoring began.

B. Trophic status and nutrients

PLEON lakes range from oligotrophic to eutrophic (Figure 21). Trophic status distribution depends on metric, suggesting that transparency in some lakes is driven by factors other than algae. For example, in 2020, TSI_{Secchi} was consistently greater than $TSI_{chlorophyll}$ and TSI_{TP} in the Twin and Walker lakes. Nitrogen availability can play a role in regulating algae growth. PLEON lakes range in summer epilimnetic TN with all lakes having <1 mg/L. 2020 TN of Twin and Walker lakes was within the PLEON range.

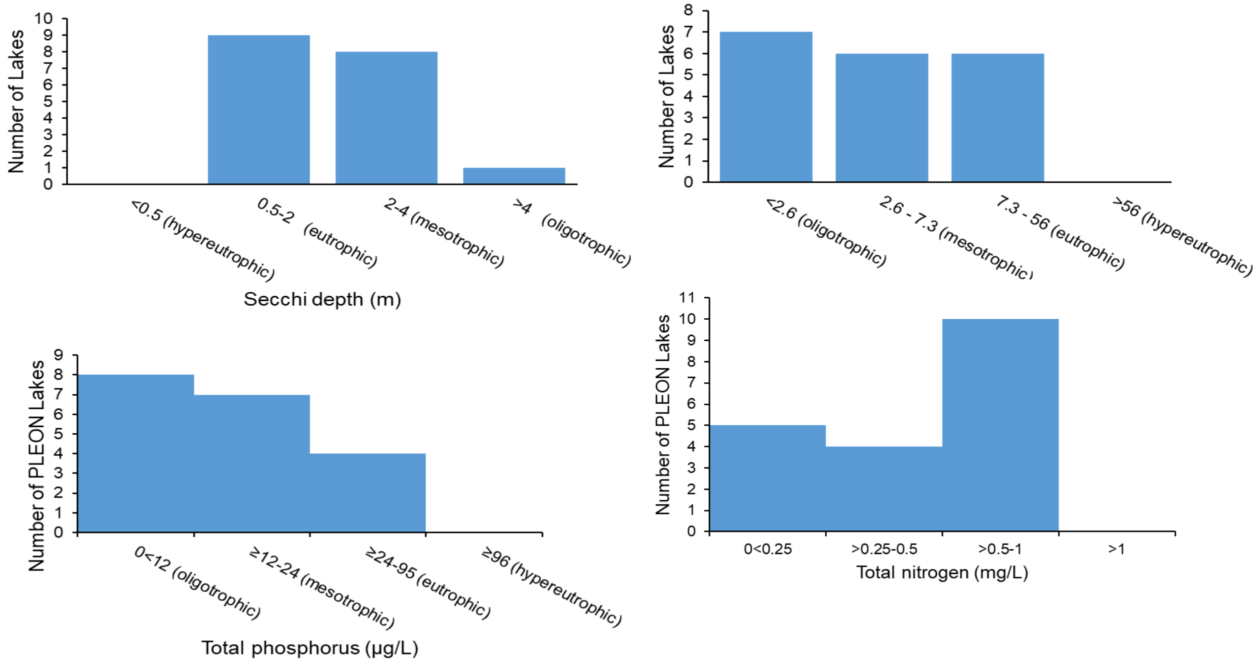

Figure 21. PLEON lakes by most recent summer Secchi depth, chlorophyll a, TP, and TN (at 0.5 m). Not all lakes are monitored for all variables each year.

D. PTOX Cyanobacteria

Nine cyanobacteria genera that can produce cyanotoxins have been found in PLEON lakes (Figure 22). *Dolichospermum* is the most common, followed by *Aphanizomenon* and *Chrysosporum*, and then by *Microcystis* and *Woronichinia*. *Dolichospermum, Aphanizomenon,* and *Chrysosporum* have been found in Twin and Walker lakes.

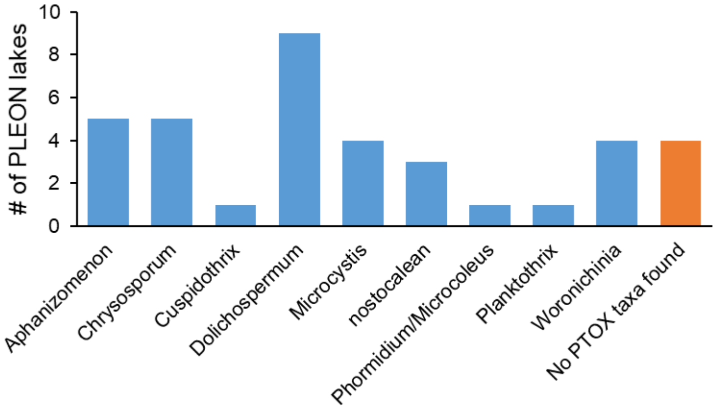

Figure 22. PTOX genera found (at least once) in Greenwater Laboratory PTOX screens of PLEON lakes from 2017-2020. Some lakes were screened on more than one date.

PLEON has conducted a total of 121 PTOX screens since May 2017. Based on the recommendation of Greenwater Laboratories, many of these samples have been tested for specific toxins (Table 13). Microcystin/nodularins are the only toxins that have been found above the minimum detection limits in these analyses. However, it is important to note that recommended testing has often been declined. Analysis of cyanotoxin concentration (Table 12) in Twin and Walker Lakes through the PA Harmful Algae Bloom Task Force program did not find detectable levels of any of the toxins analyzed from 24 August 2020 samples.

Table 13. 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	45	37	21	16	11.7	0.16-129
cylindrospermopsin	28	20	20	0	-	-
anatoxin-a	28	22	22	0	-	-
saxitoxin	27	21	21	0	-	-
homoanatoxin-a	1	1	1	0	-	-

**minimum detection limits*

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

Several findings from the Twin and Walker lakes 2020 monitoring program should be highlighted:

1. Increased productivity and phosphorus in Little Twin.

Little Twin Lake has generally had less algae, less phosphorus, and been the clearest of the three lakes since 2014 and has generally been classified as oligo-mesotrophic over this time period. However, since 2018, average summer epilimnetic phosphorus concentration has been trending upwards (following a declining trend since 2016). In 2020, Little Twin had the most phosphorus of all three lakes. Algae abundance in Little Twin also increased since 2019. Both phosphorus concentration and algae abundance has varied over the historical record (since 2014) and it is possible that these recent shifts represent more variation. Continued monitoring will show if these shifts indicate true trends.

Nutrients, including phosphorus, can fuel algae growth. There are many potential sources of nutrients that could be contributing to increased phosphorus or phosphorus variability in Little Twin (Table 14). In addition, many clear water lakes are experiencing increases in dissolved organic carbon (DOC), a phenomenon known as lake browning[3]. DOC is leached from soils and organic matter in the watershed when it rains, much as a tea bag leaches soluble organic material into a cup of water. Increased DOC in a lake can contribute to warming surface waters, deep water anoxia (see Point 2), and nutrient regeneration from sediments (see Point 2), all which can stimulate algae growth. August

2020 was the first time PLEON quantified DOC in the Twin and Walker lakes. Continued monitoring of DOC will help determine if browning may be impacting the lakes.

Table 14. Major sources and removal pathways of bioavailable P and N to lakes. Human-driven inputs are shown in red font.

P		N	
Inputs	***Removal pathways***	***Inputs***	***Removal pathways***
Watershed geology	Burial in sediments	Runoff from soil decomposition	N_2 production
Runoff from soil decomposition	Fishing/biomass removal	N fixation by cyanobacteria	Fishing/biomass removal
Leaky septic systems		Leak septic systems	
Lawn/agricultural fertilizers		Lawn/agricultural fertilizers	
Changes in land use or forest management		Changes in land use or forest management	
Remobilization from sediments		Deposition of gaseous fossil fuel byproducts	

2. Deep water hypoxia/anoxia was observed in all lakes, creating conditions for nutrient regeneration.

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].

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 of the boundary of oxygen-depleted water in Walker has been relatively stable (with some variation) since 2014 but only 2 years of data are available for Big Twin and Little Twin. Using a longer YSI probe cable will allow for complete oxygen profiles in these deep lakes over the entire summer.

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. 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. TWCWC may wish to consider collecting hypolimnion samples in the future to capture changes in nutrient regeneration.

3. Surface waters in Big and Little Twin are getting warmer.

Average summer epilimnetic temperatures in Big Twin and Little Twin have increased since 2014. This trend is statistically significant in both lakes. Many lakes around the world are warming[4]. Changing global temperatures may be playing a role but research on Pocono lakes suggests that surface warming, particularly in historically clear water lakes, is related to lake browning[3] (see Point 1). DOC can act as an insulator, trapping heat within lake water. We don’t know if increased temperature in Big and Little Twin are correlated with increasing DOC concentrations as there are no historic DOC data.

4. 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 likely due to another factor. The impacts of septic inputs may be diluted by the large size of Big Twin while exaggerated in Little Twin. The seasonal conductivity and TDS pattern (greater conductivity and more TDS in late compared to early summer) noticed in 2019 were not present in 2020.

5. pH profiles

pH is a measure of the acidity of the water using a logarithmic scale from 0 (acidic) to 14 (basic). pH of lakes depends on several factors, including the geology of the watershed, atmospheric deposition, and biological processes. Carbon dioxide concentration in the water affects pH so the processes such as photosynthesis and decomposition can impact pH. The pH of freshwater ecosystems usually range from 6-8[5].

pH of the Twin and Walker lakes were generally within this range. The pH of Walker Lake in the mid-deep waters was as low as 5.4. This is on the low end of the pH measured since 2014 in this lake (data not shown). pH can decrease with depth in stratified lakes as carbon dioxide from decomposition builds up. The pH profile in Walker shows a peak between 4-6 m, generally in the metalimnion. Point-source pollutants can also affect pH, including mine, agricultural, and wastewater runoff.

6. There is the potential for harmful algae blooms in Twin and Walker lakes.

Several lines of evidence point to the potential of harmful algae blooms (HABs) in Twin and Walker Lakes. Therefore, TWCWC may want to consider a comprehensive HABs monitoring plan.

First, there has been at least one visible algae bloom in Big Twin (Figure 23). The bloom pictured here was present on 24 August 2020.

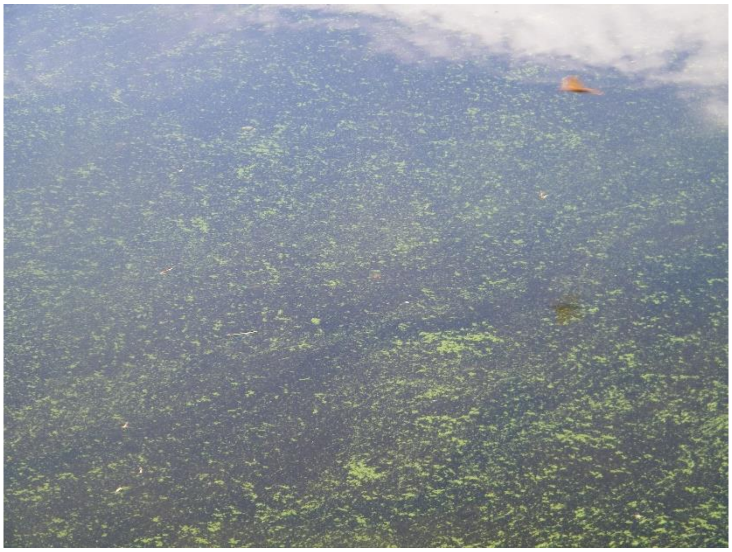

Figure 23. Possible cyanobacteria bloom near the shore of Big Twin Lake on August 24, 2020

Second, three potentially toxigenic (PTOX) cyanobacteria genera were found in the lakes with abundances high enough to prompt toxin analysis according to the program's protocols. The concentration of all toxins tested were below detection in all three lakes. The PTOX screens and toxin testing described here were conducted by the PA Harmful Algae Task Force, with PLEON acting as the sample collector. Samples were collected on 24 August 2020 and included a sample of the visible bloom in Big Twin.

Third, cyanobacteria made up substantial proportions of the algae community in Big and Little Twin lakes (34% and 59%, respectively). In Big Twin, 100% of these cyanobacteria were potentially toxigenic (capable of producing toxins) while 35% of the cyanobacteria in Little Twin were potentially toxigenic.

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

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

60-ml subsamples of water samples collected by TWCWC were frozen at -20°C until analysis for total nitrogen (TN) and total phosphorus (TP) at Lacawac Sanctuary.

Total nutrient samples were digested by adding potassium persulfate as an oxidizing reagent and heating under pressure (using a pressure cooker, 15 psi for 30 min). This Total nutrient samples were thawed and digested by adding potassium persulfate (oxidizing reagent) and heating under pressure (using a pressure cooker, 15 psi for 30 minutes). This process simultaneously converts ammonium, inorganic nitrogen (excluding N_2), and organic nitrogen to nitrate (NO_3^-) and inorganic and organic phosphorus to orthophosphate (PO_4^{-3}).

NO_3-N concentration of the digested samples was quantified using the second derivative method described by Crumpton et al. 1992. Absorbance was measured from 190-250 nm using a scanning spectrophotometer. The second derivative of the absorbance curve was calculated and the largest derivative value from 220-230 nm was used to calculate NO_3-N concentration from a standard curve.

PO_4-P concentration of the digested samples was quantified using the ascorbic acid colorimetric method with the absorbance measured at 800 nm used to calculate PO_4-P concentration using a standard curve.

D. Dissolved organic carbon (DOC)

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

E. 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 the middle of the metalimnion 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. Miami University Global Change Limnology Laboratory

PLEON DOC samples are analyzed by Erin Overholt, manager of the Global Change Limnology Laboratory (Principal Investigator: Dr. Craig Williamson) at Miami University of Ohio.

B. 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.
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4. O'Reilly, C., M. et al. 2015. Rapid and highly variable warming of lake surface waters around the globe. Geophysical Research Letters, 42:10773-10781
5. Fondriest Environmental, Inc. “pH of Water.” Fundamentals of Environmental Measurements. 19 Nov. 2013. Web. https://www.fondriest.com/environmental-measurements/parameters/water-quality/ph/.

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. Reports provided by the PA Harmful Algal Bloom Task Force

Included as separate files:

Reports from Bureau of Laboratories:

45084 Little Twin Lake

45086 Big Twin Lake

45089 Walker Lake

Reports from Greenwater Laboratories

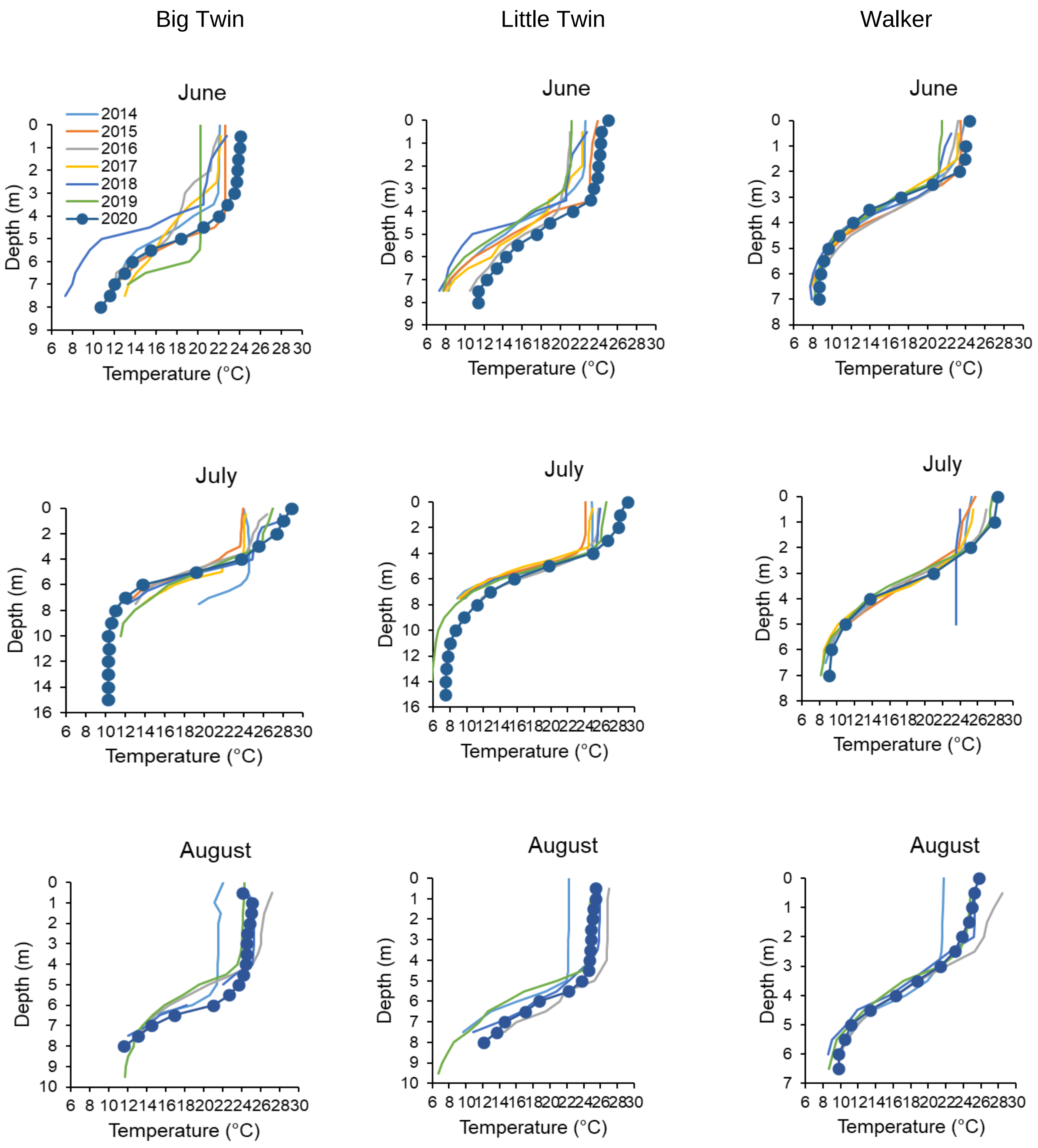
PA DEP PTOX Cyanobacteria Screen 200824_inSP_RedactedforTWCWC

PA DEP MC-STX ELISA Data 200824_RedactedforTWCWC

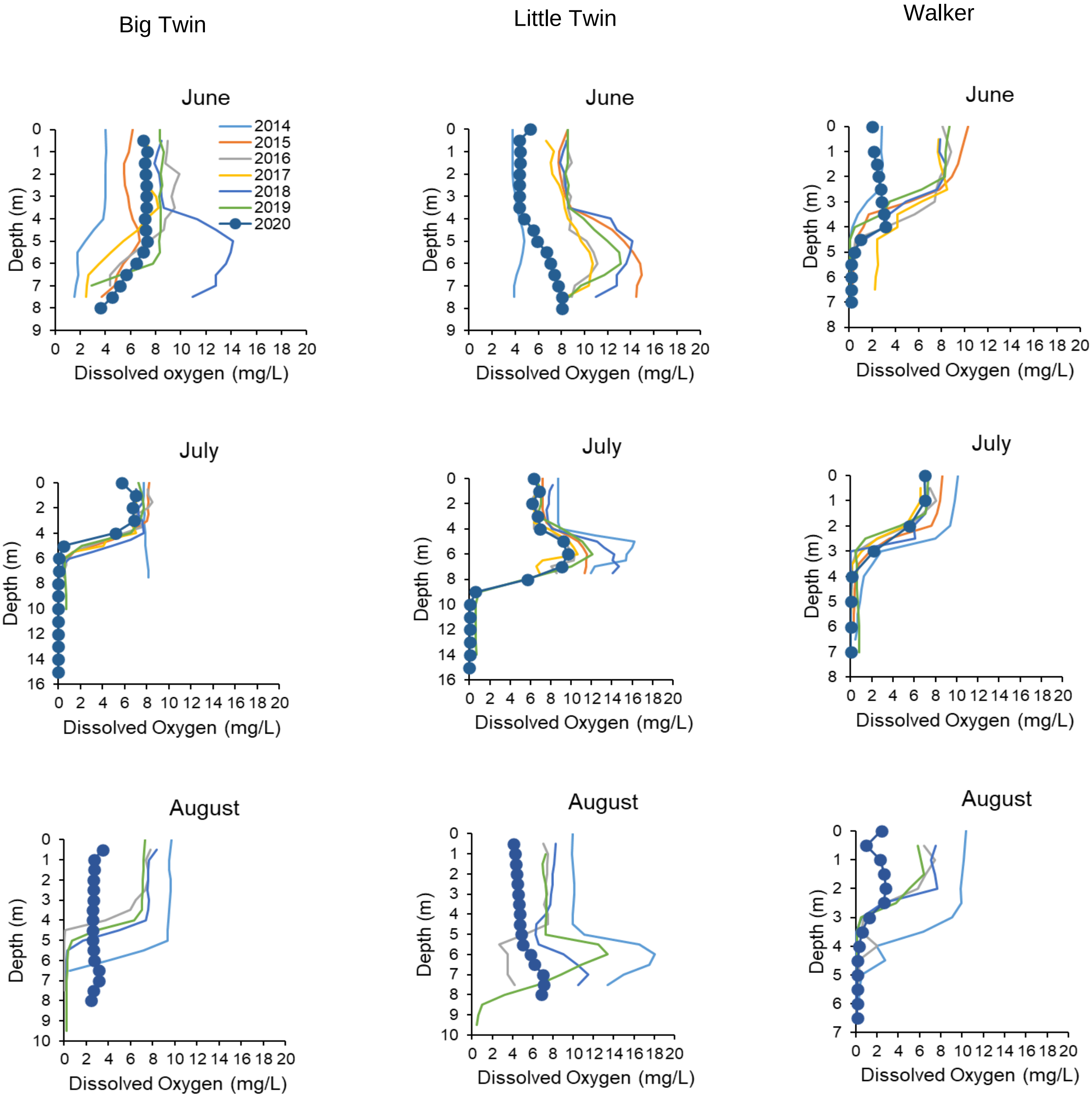
PA DEP ATX-CYN-MC-STX Report 200824_inSP_RedactedforTWCWC

Appendix VI. Supplementary data

A. Summer temperature profiles by month since 2014



B. Summer dissolved oxygen profiles by month 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
2020	81.2	81.6	81.0	ND	157.6	155.6	151.4	ND	76.9	78.9	75.5	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
2020	52.7	53.3	52.7	ND	102.7	101.4	98.1	ND	50.1	51.4	48.8	ND

Appendix VII. 2019 raw data

Attached as a separate excel file

2020_PLEON_TwinWalker_rawdata_toTWCWC