



To the Twin and Walker Creeks Watershed Conservancy

## Report of 2021 PLEON Sampling

From the Pocono Lakes Ecological Observatory Network

Beth Norman, PhD

Director of PLEON

Director of Science and Research

Lacawac Sanctuary and Biological Field Station

94 Sanctuary Rd

Lake Ariel PA 18436

## Table of Contents

I. Summary 2021: Twin and Walker Lakes at a Glance .....	4
A. Description of monitoring activities .....	4
B. Summary of water quality .....	5
II. Chemical Profiles .....	6
A. Temperature .....	6
B. Dissolved Oxygen .....	8
C. Conductivity .....	9
D. pH .....	11
E. Total Dissolved Solids .....	12
III. Water Transparency .....	13
A. Secchi depth .....	13
B. Light attenuation .....	14
IV. Chlorophyll Results .....	15
V. Nutrient Results .....	16
A. Total nitrogen .....	16
B. Total phosphorus .....	17
C. Dissolved organic carbon .....	19
VI. Plankton Communities .....	19
A. Zooplankton .....	19
B. Phytoplankton .....	21
VII. PTOX Cyanobacteria Screen .....	22
VIII. Historical Context: Twin and Walker Lakes Over Time .....	22
A. Description of historical dataset .....	22
B. Chemical profiles over time .....	22
C. Water transparency over time .....	24
D. Chlorophyll a over time .....	25
E. Nutrients over time .....	25
F. Trophic status over time .....	26
G. Zooplankton over time .....	26
H. Phytoplankton over time .....	28
I. Cyanobacteria and cyanotoxins over time .....	29

IX. Twin and Walker Lakes in the Context of the Poconos .....	31
A. Description of PLEON Lakes .....	31
B. Lake productivity .....	31
C. PTOX Cyanobacteria .....	32
X. What it all Means: Emerging Concerns for Twin and Walker Lakes .....	33
APPENDIX I: Description of Field Sampling Methods .....	35
A. Physical Profiles .....	35
B. Chlorophyll .....	35
C. Nutrients .....	35
D. Dissolved organic carbon (DOC) .....	36
E. PTOX screening and cyanotoxin analysis .....	36
F. Plankton community analysis .....	36
Appendix II: Literature Cited.....	37
Appendix III: Glossary.....	37
Appendix IV. Greenwater Laboratory Reports.....	39
Appendix V. Summer monthly profile data .....	39

## I. Summary 2021: 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 2021. The lakes were part of a research collaboration between PLEON and Dr. Sarah Princiotta of Penn State, Schuylkill, funded by the Pennsylvania Water Resources Research Center (PAWRRC). Available data from this study are included in this report. Remaining data will be provided when available. PLEON collected a sample for potentially toxic cyanobacteria screening from Big Twin Lake on June 2, 2021.

**Table 1: Summary of 2021 monitoring. Variables in red were part of the PAWRRC project. First date indicates sampling of Twin Lakes, second date indicates sampling of Walker.**

	<b>Variables Monitored</b>	<b>Crew</b>
27 June	<ul style="list-style-type: none"> <li>• Profiles: temperature, dissolved oxygen, conductivity, pH, dissolved solids</li> <li>• Secchi Depth</li> <li>• Chlorophyll a (0.5 m, composite)</li> <li>• Total N, Total P, dissolved organic carbon concentration (0.5 m, composite)</li> </ul>	Collection: TWCWC Analysis: PLEON
17 July 19 July	<ul style="list-style-type: none"> <li>• Profiles: temperature, dissolved oxygen, conductivity, pH, dissolved solids</li> <li>• Secchi Depth</li> <li>• Chlorophyll a (0.5 m, composite)</li> <li>• Total N, Total P, dissolved organic carbon concentration (0.5 m, composite)</li> </ul>	Collection: TWCWC Analysis: PLEON
20 July 22 July	<ul style="list-style-type: none"> <li>• Profiles: temperature, dissolved oxygen, conductivity, pH, light</li> <li>• Secchi Depth</li> <li>• Composite zooplankton community (Twin Lakes only)</li> <li>• Composite phytoplankton community (Twin Lakes only)</li> <li>• Chlorophyll a at 0.5 m</li> <li>• Total N and total P at 0.5 m*</li> <li>• Phycocyanin at 0.5 m*</li> <li>• Cyanotoxin concentration at 0.5 m*</li> <li>• Phytoplankton composition at 0.5 m*</li> </ul>	Meghan Corridoni (PLEON intern) Matthew Simms (PLEON intern)
26 Aug 21 Aug	<ul style="list-style-type: none"> <li>• Profiles: temperature, dissolved oxygen, conductivity, pH, dissolved solids</li> <li>• Secchi Depth</li> <li>• Chlorophyll a (0.5 m, composite)</li> <li>• Total N, Total P, dissolved organic carbon concentration (0.5 m, composite)</li> </ul>	Collection: TWCWC Analysis: PLEON
31 Aug	<ul style="list-style-type: none"> <li>• Profiles: temperature, dissolved oxygen, conductivity, pH, light</li> <li>• Secchi Depth</li> <li>• Chlorophyll a at 0.5 m</li> <li>• Total N and total P at 0.5 m*</li> <li>• Phycocyanin at 0.5 m*</li> <li>• Cyanotoxin concentration at 0.5 m*</li> <li>• Phytoplankton composition at 0.5 m*</li> </ul>	Sarah Princiotta (PAWRRC lead) Kayla Baney (PAWRRC intern)
25 Sep	<ul style="list-style-type: none"> <li>• Profiles: temperature, dissolved oxygen, conductivity, pH, light</li> <li>• Secchi Depth</li> <li>• Chlorophyll a at 0.5 m</li> <li>• Total N and total P at 0.5 m*</li> <li>• Phycocyanin at 0.5 m*</li> <li>• Cyanotoxin concentration at 0.5 m*</li> <li>• Phytoplankton composition at 0.5 m*</li> </ul>	Sarah Princiotta (PAWRRC lead) PAWRRC intern

\*Analysis pending

## B. Summary of water quality

**Table 2: Summary of Big Twin Lake in 2021**

	2 Jun	27 June	17 July	20 July	26 Aug	31 Aug	25 Sep
Thermally stratified?*	—	YES	YES	YES	YES	UD	UD
Epilimnion depth (m)	—	3	3	8	4	4	>5
Metalimnion depth (m)*	—	7	8	12	9	UD	UD
Secchi depth (m)	—	3.4	2.85	2	2.7	2	2
Vertical extinction coefficient (k)	—	—	—	0.75	—	0.93	1.15
Z <sub>10%</sub> (m)	—	—	—	3.07	—	2.48	2.00
Z <sub>1%</sub> (m)	—	—	—	6.14	—	4.96	4.00
Mean hypolimnetic DO (mg/L)	—	0.43	0.04	0.04	0.03	—	—
Epilimnetic chlorophyll concentration (µg/L)	—	1.40	1.26	2.05	1.63	2.93	1.89
Epilimnetic TN (mg/L)*	—	0.44	0.51	Pending <sup>***</sup>	BD	Pending <sup>***</sup>	Pending <sup>***</sup>
Epilimnetic TP (µg/L)*	—	BD	5.99	Pending <sup>***</sup>	9.43	Pending <sup>***</sup>	Pending <sup>***</sup>
TSI <sub>secchi</sub>	—	42.4	44.9	50.0	45.7	50.0	50.0
TSI <sub>chlorophyll</sub>	—	33.9	32.9	37.6	35.4	41.1	36.8
TSI <sub>TP</sub>	—	—	29.6	Pending <sup>***</sup>	36.0	Pending <sup>***</sup>	Pending <sup>***</sup>
Trophic classification**	—	OLIGO	OLIGO	OLIGO	OLIGO	MESO	OLIGO
PTOX cyanobacteria found?	YES	—	—	—	—	—	—
Toxin testing recommended?	YES	—	—	—	—	—	—

\*UD = undetermined due to sampling depth, BD = below detection depends on metric. OLIGO = oligotrophic, MESO = mesotrophic. \*\*according to TSI<sub>chlorophyll</sub>, status depends on metric. \*\*\*Part of PAWRRC project, data pending.

**Table 3: Summary of Little Twin Lake in 2021**

	27 June	17 July	20 July	26 Aug	31 Aug	25 Sep
Thermally stratified?*	YES	YES	YES	YES	UD	UD
Epilimnion depth (m)	2	2	3	4	4	>5
Metalimnion depth (m)*	10	9	9	10	UD	UD
Secchi depth (m)	5	3.6	3.5	2.8	3.75	2.5
Vertical extinction coefficient (k)	—	—	0.55	—	0.84	0.83
Z <sub>10%</sub> (m)	—	—	4.21	—	2.73	2.76
Z <sub>1%</sub> (m)	—	—	8.42	—	5.46	5.52
Mean hypolimnetic DO (mg/L)	0.38	0.19	0.11	0.22	—	—
Epilimnetic chlorophyll concentration (µg/L)	—	—	0.91	2.53	2.04	4.21
Epilimnetic TN (mg/L)	0.73	0.35	Pending <sup>***</sup>	0.11	Pending <sup>***</sup>	Pending <sup>***</sup>
Epilimnetic TP (µg/L)*	8.60	BD	Pending <sup>***</sup>	16.3	Pending <sup>***</sup>	Pending <sup>***</sup>
TSI <sub>secchi</sub>	36.8	41.5	41.9	45.2	41.0	46.8
TSI <sub>chlorophyll</sub>	—	—	29.7	39.7	37.6	44.7
TSI <sub>TP</sub>	34.8	—	Pending <sup>***</sup>	43.8	Pending <sup>***</sup>	Pending <sup>***</sup>
Trophic classification**	OLIGO <sup>†</sup>	MESO <sup>†</sup>	OLIGO	OLIGO	OLIGO	MESO

\*UD = undetermined due to sampling depth, BD = below detection depends on metric. OLIGO = oligotrophic, MESO = mesotrophic. \*\*according to TSI<sub>chlorophyll</sub>, status depends on metric. \*\*\*Part of PAWRRC project, data pending. <sup>†</sup>according to TSI<sub>Secchi</sub>, TSI<sub>chlorophyll</sub> not available

**Table 4: Summary of Walker Lake in 2021**

	27 June	19 July	22 July	21 Aug	31 Aug	25 Sep
<b>Thermally stratified?*</b>	YES	YES	YES	YES	UD	UD
<b>Epilimnion depth (m)</b>	2	1.5	2	1.5	>1.5	2.5
<b>Metalimnion depth (m)*</b>	4.5	5.5	5.5	5	UD	UD
<b>Secchi depth (m)</b>	1.3	1.3	1.5	1.2	1	1.15
<b>Vertical extinction coefficient (k)</b>	—	—	—	—	1.95	1.71
<b>Z<sub>10%</sub> (m)</b>	—	—	—	—	1.18	1.35
<b>Z<sub>1%</sub> (m)</b>	—	—	—	—	2.37	2.70
<b>Mean hypolimnetic DO (mg/L)</b>	0.17	0.12	—	0.15	—	—
<b>Epilimnetic chlorophyll concentration (µg/L)</b>	8.47	—	4.53	10.5	4.15	5.31
<b>Epilimnetic TN (mg/L)</b>	0.90	0.41	Pending <sup>***</sup>	0.53	Pending <sup>***</sup>	Pending <sup>***</sup>
<b>Epilimnetic TP (µg/L)</b>	15.8	8.63	Pending <sup>***</sup>	26.6	Pending <sup>***</sup>	Pending <sup>***</sup>
<b>TSI<sub>Secchi</sub></b>	56.2	56.2	54.2	57.4	60.0	58.0
<b>TSI<sub>chlorophyll</sub></b>	51.6	—	45.4	53.6	44.5	47.0
<b>TSI<sub>TP</sub></b>	43.3	34.8	Pending <sup>***</sup>	50.8	Pending <sup>***</sup>	Pending <sup>***</sup>
<b>Trophic classification**</b>	EU	EU <sup>†</sup>	MESO	EU	MESO	MESO

\*UD = undetermined due to sampling depth \*\*according to TSI<sub>chlorophyll</sub>, status depends on metric. EU = eutrophic, MESO = mesotrophic. \*\*\*Part of PAWRRC project, data pending. †according to TSI<sub>Secchi</sub>, TSI<sub>chlorophyll</sub> not available

## II. Chemical Profiles

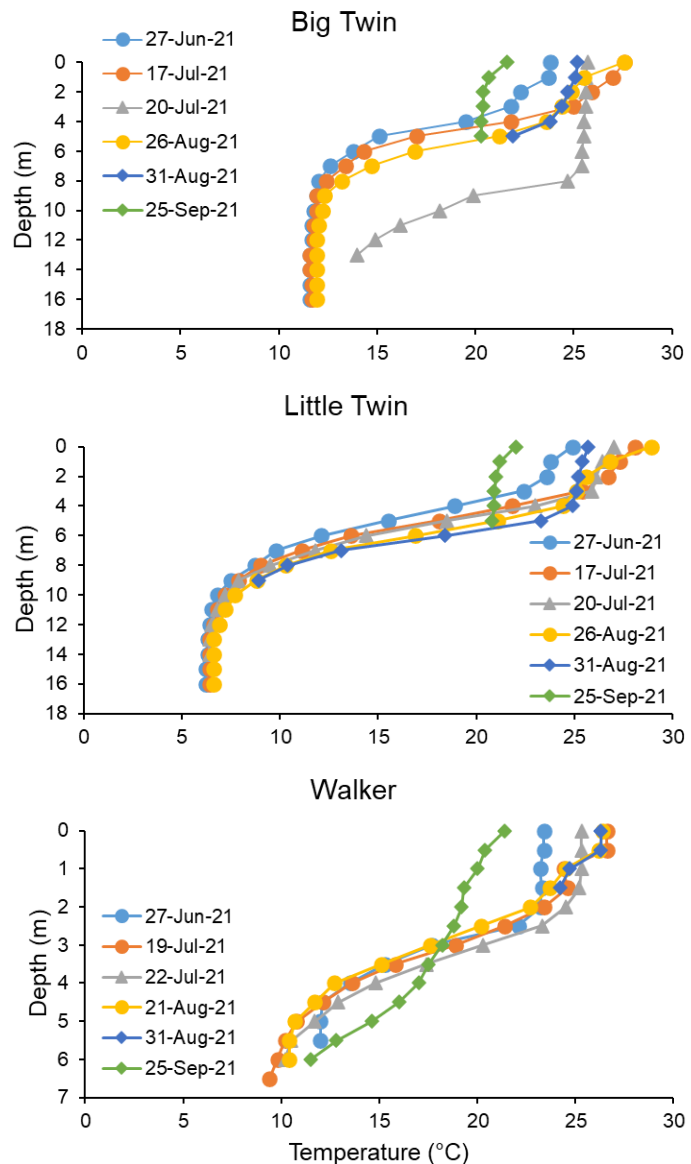
### A. Temperature

Big Twin was thermally stratified during the 27 June, 17 July, 20 July, and 26 Aug samplings (Figure 1). Incomplete depth profiles were taken during the 31 Aug and 25 Sep PAWRRC samplings so stratification could not be verified on these dates. The epilimnion, or the well-mixed surface layer, extended to 3 m, 3 m, 8 m, 4 m, and 4 m during the 27 June, 17 July, 20 July, 26 Aug, and 31 Aug sampling, respectively. The epilimnion was deeper than 5 m on the 25 September sampling. The deep epilimnion found on 20 July is puzzling, particularly as it differs so starkly from the profile taken 3 days earlier. It is possible that the moderate wind conditions on 20 July caused the probe to descend through the water column at an angle, artificially extending the epilimnion.

The average epilimnetic temperature ( $\pm$  standard deviation) in Big Twin was 22.9 °C ( $\pm$ 1.0) on 27 June, 26.4 °C ( $\pm$ 1.16) on 17 July, 25.4 °C ( $\pm$ 0.30) on 20 July, 25.2 °C ( $\pm$ 1.51) on 26 Aug, 24.6 °C ( $\pm$ 0.57) on 31 Aug, and 20.6 °C ( $\pm$ 0.50) on 25 Sep. The metalimnion, or middle layer of rapid temperature change, extended to 7 m, 8 m, 12 m, and 9 m during the 27 June, 17 July, 20 July, and 26 Aug sampling, respectively. The depth of the metalimnion was undetermined during the last two samplings.

Little Twin was thermally stratified during all samplings with the exception of 31 Aug and 25 Sep when the incomplete depth profiles prevented delineation (Figure 1). The epilimnion extended to 2 m, 2 m, 3 m, 4 m, and 4 m during the 27 June, 17 July, 20 July, 26 Aug, and 31 Aug sampling, respectively. The epilimnion was deeper than 5 m on the 25 September sampling. The average epilimnetic temperature ( $\pm$  standard deviation) in Little Twin was 24.1 °C ( $\pm$ 0.70) on 27 June, 27.4 °C ( $\pm$ 0.70) on 17 July, 26.4 °C ( $\pm$ 0.48) on 20 July, 26.2 °C ( $\pm$ 1.76) on 26 Aug, 25.3 °C ( $\pm$ 0.30) on 31 Aug, and 21.1 °C ( $\pm$ 0.45) on 25 Sep. The metalimnion extended to 10 m, 9 m, 9 m, and 10 m during the 27 June, 17 July, 20 July, and 26 Aug sampling, respectively. The depth of the metalimnion was undetermined during the last two samplings.

Like the Twin lakes, Walker was thermally stratified during all samplings, excluding 31 Aug and 25 Sep as explained above (Figure 1). The epilimnion extended to 2 m, 1.5 m, 2 m, and 1.5 m during the 27 June, 19 July, 22 July, and 21 Aug samplings, respectively. The epilimnion was deeper than 1.5 m during the 31 Aug sampling and extended to 2.5 m during the 25 Sep sampling. The average epilimnetic temperature ( $\pm$  standard deviation) in Walker was 23.3 °C ( $\pm$ 0.10) on 27 June, 25.6 °C ( $\pm$ 1.23) on 19 July, 25.1 °C ( $\pm$ 0.35) on 22 July, 25.2 °C ( $\pm$ 1.31) on 21 Aug, 25.4 °C ( $\pm$ 1.09) on 31 Aug, and 19.9 °C ( $\pm$ 0.95) on 25 Sep. The metalimnion extended to 4.5 m, 5.5 m, 5.5 m, and 5 m during the 27 June, 19 July, 22 July, and 21 Aug sampling, respectively. The depth of the metalimnion was undetermined during the last two samplings.



**Figure 1: Temperature profiles of TWCWC lakes in 2021. Note the different sampling dates among panels and differences in Y axis scale. Circles, triangles, and diamonds denote data collected by TWCWC, PLEON, and the PAWRRC project, respectively.**

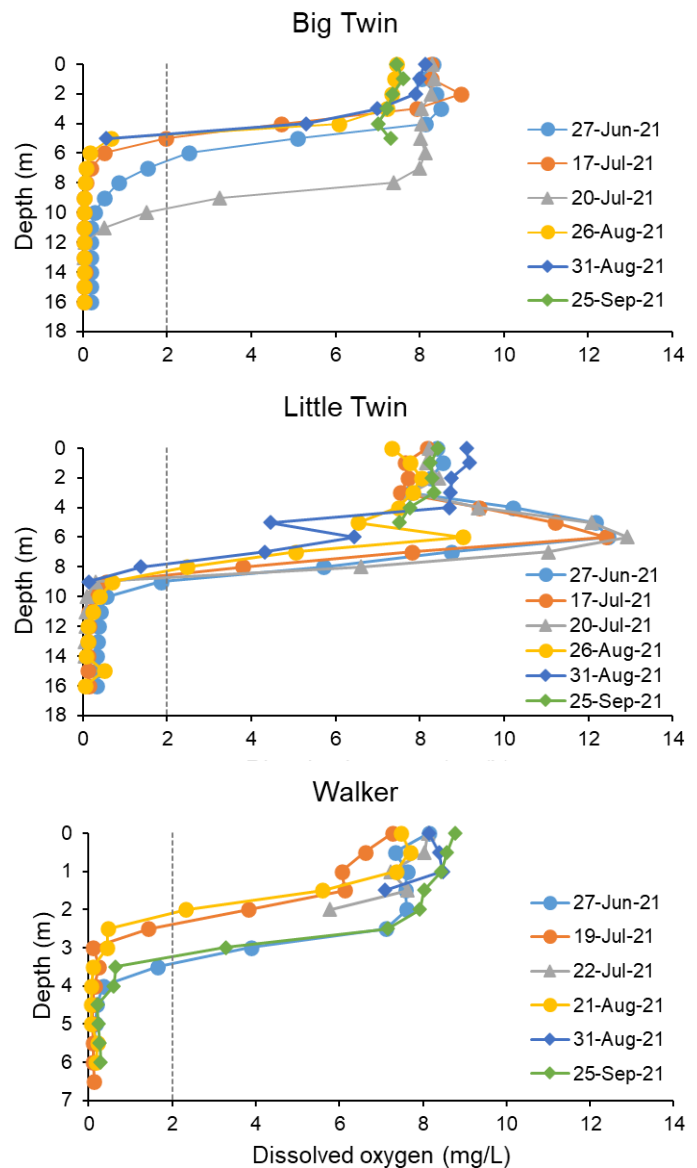
Thermal stratification of deep lakes is expected in the Pocono region as the surface water is heated by the sun and the deeper water remains cool. Thermal stratification breaks down in the fall as surface waters cool and lakes “turnover”, or the layers mix. The initial stages of this process is evident in the cooler epilimnetic temperatures of the 25 September temperature profile in the Twin lakes. The September temperature profile in Walker suggests Walker was further along this mixing process with a deep epilimnion that is almost indistinct from the metalimnion.

### B. Dissolved Oxygen

Big Twin was oxygenated through the epilimnion during all 2021 samplings (Figure 2). Dissolved oxygen (DO) concentration declined through the metalimnion during all samplings with the exception of 25 September.

Average DO concentration in the hypolimnion, or deep water, was 0.43 mg/L, 0.04 mg/L, 0.04 mg/L, and 0.03 mg/L during the 27 June, 17 July, 20 July, and 26 Aug sampling, respectively. Hypolimnetic DO concentration was not measured on 31 Aug or 25 Sep. The depth at which DO concentration was below 2 mg/L, the threshold for oxygen depletion, was 7 m, 5 m, 10 m, 5 m, and 5 m on 27 June, 17 July, 20 July, 26 Aug, and 31 Aug, respectively. The depth of oxygen depletion was greater than 5 m on 25 Sep.

Little Twin was also oxygenated through the epilimnion during all 2021 samplings (Figure 2). The maximum dissolved oxygen (DO) concentration was detected in the metalimnion during 27 June, 17 July, 20 July, and 26 August with peak DO concentrations, found at 6 m, of 12.5 mg/L, 12.4 mg/L, 12.9 mg/L, and 9.0 mg/L, respectively.



**Figure 2: Dissolved oxygen profiles of TWCWC lakes in 2021. Note the different sampling dates among panels and differences in Y axis scale. Circles, triangles, and diamonds denote data collected by TWCWC, PLEON, and the PAWRRC project, respectively. Dashed lines show threshold of oxygen depletion (2 mg/L).**



DO concentration declined at depths below these maxima. Average DO concentration in the hypolimnion was 0.38 mg/L on 27 June, 0.19 mg/L on 17 July, 0.11 mg/L on 20 July, and 0.22 mg/L on 26 August with oxygen depletion occurring at 9 m on these dates. Hypolimnetic DO concentration was not measured on 31 Aug or 25 Sep but the water column was anoxic at 8 m on 25 Aug.

As in the Twin lakes, Walker Lake was oxygenated through the epilimnion (Figure 2). DO concentration declined through the metalimnion on all sampling dates. Average hypolimnetic DO concentration was 0.17 mg/L on 27 June, 0.12 mg/L on 19 July, and 0.15 mg/L on 21 Aug with oxygen depletion occurring at 3.5 m, 2.5 m, and 2.5 m, respectively. Hypolimnetic DO concentration was not measured on 22 July, 31 Aug or 25 Sep but the water column was anoxic at 3.5 m on 25 Sep.

The DO profiles observed in Twin and Walker lakes are typical. DO concentration is often high in the epilimnion due to diffusion of oxygen across the surface of the lake as well as the abundance of algae in this warm, typically well-lit layer. Algae produce oxygen as a byproduct of photosynthesis. DO peaks in the metalimnion (sometimes referred to as metalimnetic oxygen maxima) can occur when algae congregate in the middle depths. This is common in clear water lakes, such as Little Twin, where metalimnetic waters still have plenty of light for photosynthesis but less of the harmful ultraviolet wavelengths. This maximum was not present in Little Twin on the later sampling dates, possibly due to the deepening epilimnion and initial stages of mixing or to the lingering effects of Hurricane Henri.

Oxygen depletion is common in the hypolimnion (as seen in all three TWCWC lakes) where decomposition of organic matter in the water and lake sediments removes oxygen and the lack of light prohibits photosynthesis. The hypolimnion often remains hypoxic until thermal stratification breaks down and the lake layers mix.

### C. Conductivity

Conductivity in Big Twin was generally stable through the epilimnion and increased through the deeper waters (Figure 3). The exception was the 20 July sampling when conductivity was fairly stable through the water column (but see comments in Section II.A. regarding this profile). Conductivity in the surface waters was ~10  $\mu\text{S}/\text{cm}$  less on 25 Sep compared to the other sampling dates. Conductivity ranged from 78.6-108.4  $\mu\text{S}/\text{cm}$  on 27 June, from 78.2-159.5  $\mu\text{S}/\text{cm}$  on 17 July, from 67.1-78.3  $\mu\text{S}/\text{cm}$  on 20 July, from 77.0-118.1  $\mu\text{S}/\text{cm}$  on 26 Aug, from 73.5-77.2  $\mu\text{S}/\text{cm}$  on 31 Aug, and from 65.8-77.4  $\mu\text{S}/\text{cm}$  on 25 Sep. The later two dates only include readings from the surface to 5 m. The highest conductivity was generally recorded near the sediments.

Conductivity in Little Twin was also stable through the epilimnion but was greater than that of Big Twin by ~65  $\mu\text{S}/\text{cm}$  (Figure 3). Conductivity profiles in deeper water depended on date: conductivity on 27 June, 17 Jul, and 26 Aug generally increased through the deep waters while conductivity on 20 July decreased to a depth of ~9 m and then increased toward the sediments. Conductivity in the surface waters was ~20

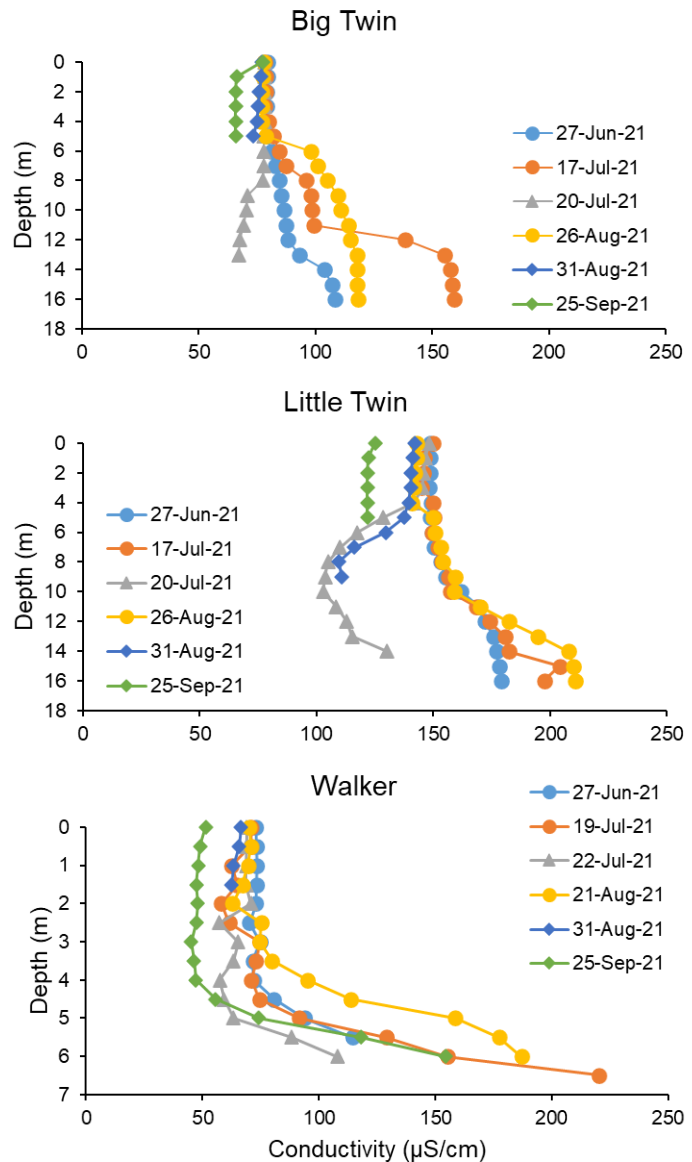
$\mu\text{S}/\text{cm}$  less on 25 Sep compared to the other sampling dates.

Conductivity ranged from 148.3-179.2  $\mu\text{S}/\text{cm}$  on 27 June, from 145.0-204.1  $\mu\text{S}/\text{cm}$  on 17 July, from 102.8-148.4  $\mu\text{S}/\text{cm}$  on 20 July, from 141.0-210.6  $\mu\text{S}/\text{cm}$  on 26 Aug, from 109.4-141.9  $\mu\text{S}/\text{cm}$  on 31 Aug, and from 121.8-125.1  $\mu\text{S}/\text{cm}$  on 25 Sep. Profiles extend to 9 m on 31 Aug and to 5 m on 25 Sep.

Conductivity of Walker was more similar to that of Big Twin, both in magnitude and over depth (Figure 3). Conductivity in Walker was generally stable through the epilimnion and increased with depth through the deeper layers.

Conductivity in the epilimnion was  $\sim 20$   $\mu\text{S}/\text{cm}$  less on 25 Sep compared with the other dates. Conductivity ranged from 70.1-114.3  $\mu\text{S}/\text{cm}$  on 27 June, from 58.1-220.4  $\mu\text{S}/\text{cm}$  on 19 July, from 57.3-107.9  $\mu\text{S}/\text{cm}$  on 22 July, from 62.8-187.0  $\mu\text{S}/\text{cm}$  on 21 Aug, from 24.2-26.3  $\mu\text{S}/\text{cm}$  on 31 Aug, and from 45.1-154.5 on 25 Sep. The 31 Aug profile extended to 1.5 m.

Conductivity is a measure of the amount of ions, or charged particles, in the water which come from dissolved compounds. Lake conductivity responds to several factors including underlying geology, runoff, point-source inputs, precipitation, evaporation, and in-lake productivity. Increased conductivity near the sediments in TWCWC lakes may be a result of the increased biological activity at the water sediment interface. The lower conductivity observed in September may be a seasonal effect or a result of dilution from the precipitation of Hurricane Ida (late summer).



**Figure 3: Conductivity profiles of TWCWC lakes in 2021. Note the different sampling dates among panels and differences in Y axis scale. Circles, triangles, and diamonds denote data collected by TWCWC, PLEON, and the PAWRRC project, respectively.**

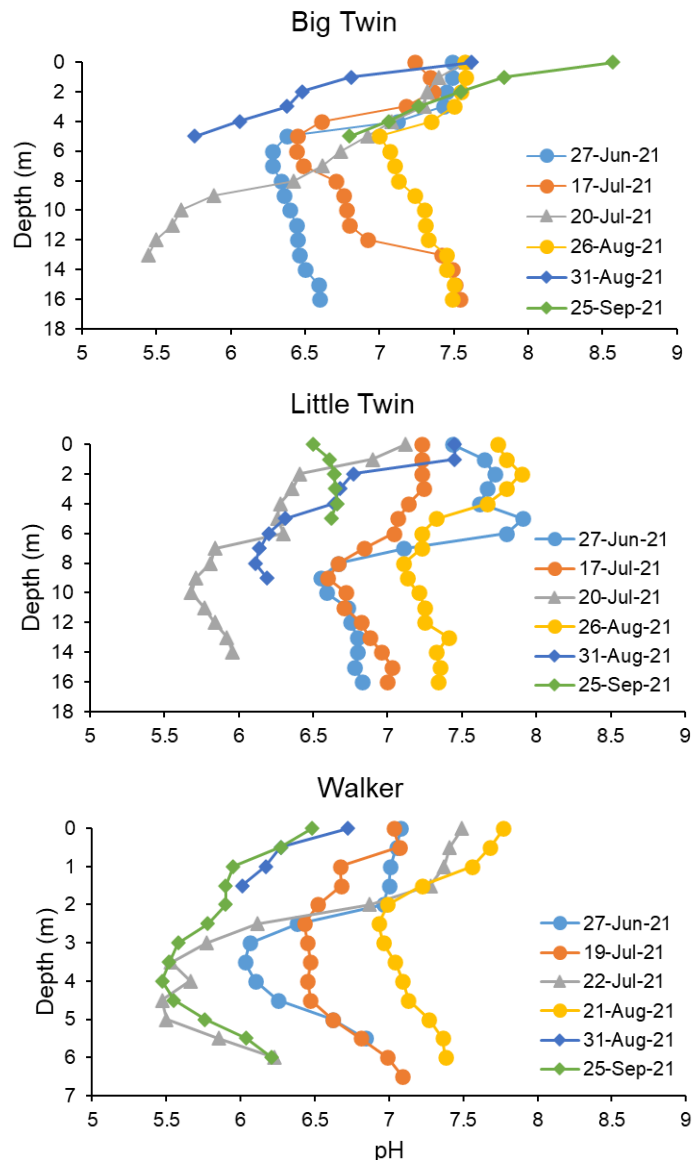
#### D. pH

pH in Big Twin was generally stable in the epilimnion and decreased to a depth of ~6-8 m (Figure 4). The 31 Aug and 25 Sep samplings were exceptions; pH declined from the surface through the profile on these dates. pH generally slightly increased through the deep water, except on 20 July when it continued to decline. pH in Big Twin ranged from 6.28-7.49 on 27 June, from 6.44-7.54 on 17 July, from 5.45-7.58 on 20 July, from 7.00-7.58 on 26 Aug, from 5.76-7.62 on 31 Aug, and from 6.80-8.57 on 25 Sep.

pH in Little Twin was somewhat variable in the epilimnion and was generally lower in the hypolimnion compared to the surface waters (Figure 4). pH in Little Twin ranged from 6.55-7.91 on 27 June, from 6.60-7.24 on 17 July, from 5.68-7.12 on 20 July, from 7.11-7.90 on 26 Aug, from 6.11-7.45 on 31 Aug, and from 6.50-6.66 on 25 Sep. Profiles extend to 9 m on 31 Aug and to 5 m on 25 Sep.

pH in Walker generally decreased from the surface to a depth of ~3-4 m and then increased toward the sediments (Figure 4). pH in Walker ranged from 6.03-7.08 on 27 June, from 6.43-7.09 on 19 July, from 5.47-7.49 on 22 July, from 6.93-7.77 on 21 Aug, from 6.01-6.72 on 31 Aug, and from 5.47-6.48 on 25 Sep. The 31 Aug profile extended to 1.5 m.

pH is a measure of the acidity of water with a logarithmic scale ranging from 0 (very acidic) to 14 (very basic). Freshwater ecosystems are usually pH neutral, typically ranging from 6-9<sup>1</sup>. pH in the TWCWC lakes was generally within this range, although sometimes a little more acidic. Several factors affect water pH, including geology, precipitation, runoff, point-source inputs, and carbon dioxide. Carbon dioxide, a



**Figure 4: pH profiles of TWCWC lakes in 2021. Note the different sampling dates among panels and differences in Y axis scale. Circles, triangles, and diamonds denote data collected by TWCWC, PLEON, and the PAWRRC project, respectively.**

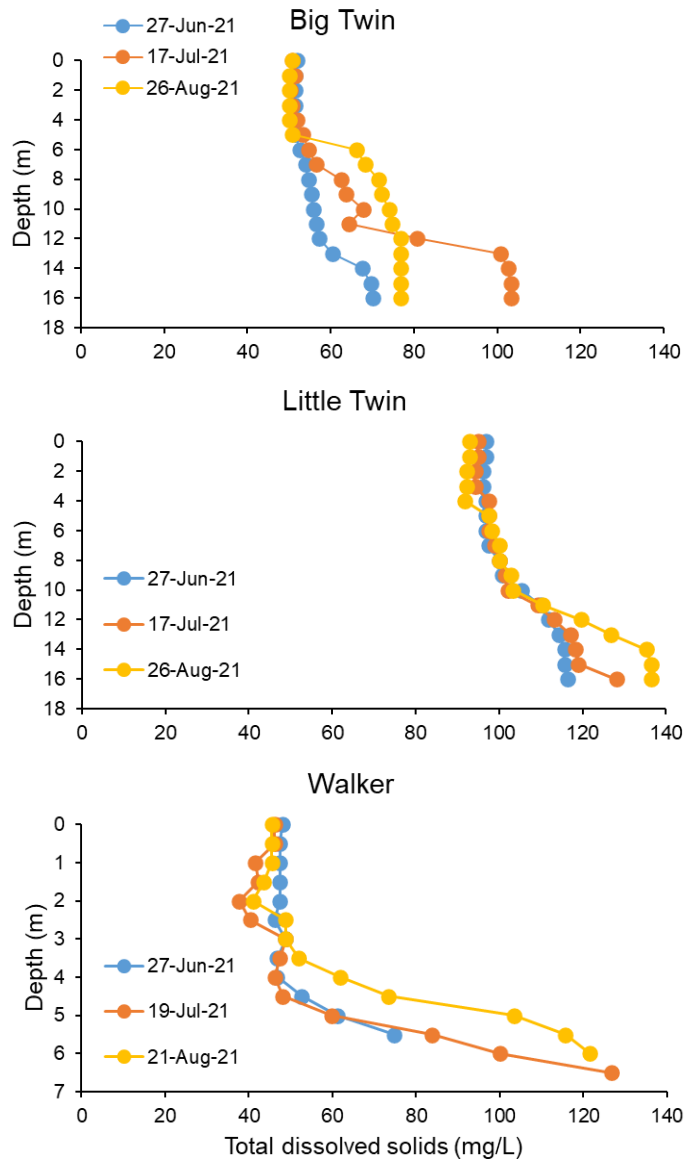
biprodukt of decomposition, forms carbonic acid in water. Decomposition in the hypolimnion can contribute to the declining pH through depth in stratified lakes<sup>2</sup>. This was seen in some, but not all, TWCWC profiles. pH readings on 31 Aug and 25 Sep tended to be lower than the other sampling dates. This may be due to precipitation inputs from Hurricanes Henri and Ida as rain tends to have a lower pH than lake water<sup>2</sup>.

#### E. Total Dissolved Solids

Total dissolved solids (TDS) concentration in Big Twin was stable at ~50 mg/L through the epilimnion during the 27 June, 17 July, and 26 Aug sampling (TDS concentration was not measured on other dates; Figure 5). TDS concentration increased with depth through the metalimnion into the deep waters. TDS concentration in Big Twin ranged from 51.4-79.2 mg/L on 27 June, from 50.4-103.4 mg/L on 17 July, and from 50.1-76.7 mg/L on 26 Aug.

TDS concentration in Little Twin was greater than that of Big Twin, ranging from ~92-94 mg/L through the epilimnion during the 27 June, 17 July, and 26 Aug sampling (Figure 5). TDS concentration increased slightly through the metalimnion and more dramatically through the hypolimnion. TDS concentration in Little Twin ranged from 96.2-116.4 mg/L on 27 June, from 94.3-128.1 mg/L on 17 July, and from 91.7-136.5 on 26 Aug.

TDS concentration in Walker was more similar to Big Twin, ranging from ~37-47 mg/L through the epilimnion into the metalimnion during the 27 June, 19 July, and 21 Aug samplings (Figure 5). TDS concentration increased with depth through the hypolimnion. TDS concentration in



**Figure 5: TDS profiles of TWCWC lakes in 2021. Note the different sampling dates among panels and differences in Y axis scale. All profiles were collected by TWCWC.**

Walker ranged from 46.2-74.8 mg/L on 27 June, from 37.7-126.8 mg/L on 19 July, and from 41.0-121.6 mg/L on 21 Aug.

TDS concentration is the amount of dissolved particles, including minerals, salts, and ions. Factors affecting TDS include geology, precipitation, runoff, and activities within the watershed such as pesticide and fertilizer application, use of road salts, and faulty septic systems. TDS concentration in freshwaters usually ranges from 50-250 mg/L<sup>3</sup>. TDS concentration in TWCWC lakes were within this range.

### III. Water Transparency

#### A. Secchi depth

Secchi depth is a measure of water transparency and is defined as the depth at which an 8-inch diameter black and white disk lowered straight down into the water disappears from view. Lakes with clear water have deeper Secchi depths than those with more murky or dark water. Several factors influence water transparency such as the amount of suspended particles (including algae) and the amount and color of dissolved compounds. Secchi depth can be used to calculate Carlson’s Trophic State Index (TSI) according to the following equation<sup>4</sup>:

$$TSI_{secchi} = 60 - 16.41 \times \ln (Secchi\ depth)$$

Secchi depth in Big Twin was 3.4 m, 2.9 m, 2 m, 2.7 m, 2 m, and 2 m during the 27 June, 17 July, 20 July, 26 Aug, 31 Aug, and 25 Sep sampling, respectively. TSI<sub>Secchi</sub> of Big Twin across these dates was 42.4, 44.9, 50.0, 45.7, 50.0, and 50.0, respectively, classifying Big Twin as mesotrophic or mesotrophic/eutrophic (Table 5).

Secchi depth in Little Twin was generally deeper than in Big Twin across these dates, reading 5 m, 3.6 m, 3.5 m, 2.8 m, 3.75 m, and 2.5 m, respectively. TSI<sub>Secchi</sub> of Little Twin across dates was 36.8, 41.5, 41.9, 45.2, 41.0, and 46.8, respectively, classifying Little Twin as oligotrophic on 27 June and mesotrophic on other dates (Table 5).

Secchi depth in Walker was more shallow than both Twin lakes, reading 1.3 m, 1.3 m, 1.5 m, 1.2 m, 1 m, and 1.15 m on 27 June, 19 July, 22 July, 21 Aug, 31 Aug, and 25 Sep, respectively. TSI<sub>Secchi</sub> of Walker across these dates was 56.2, 56.2, 54.2, 57.4, 60.0, and 58.0, respectively, classifying Walker as eutrophic (Table 5).

**Table 5: Trophic classification description**

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

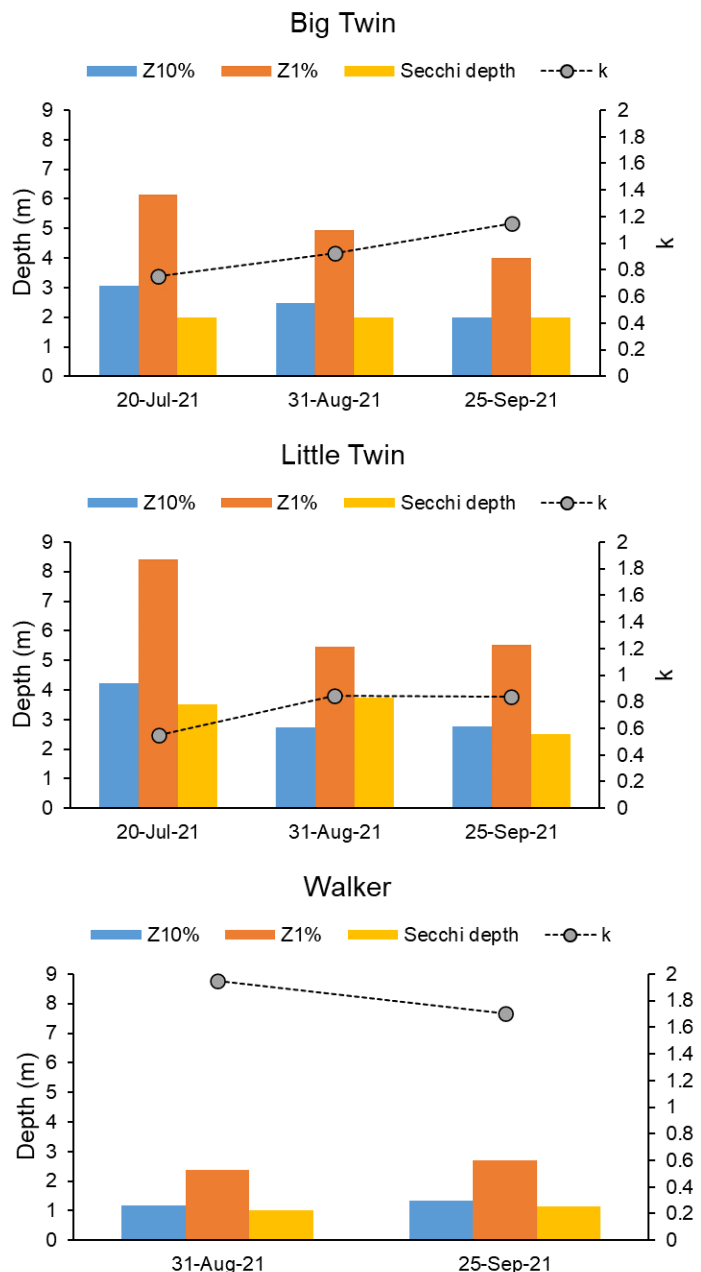
## B. Light attenuation

Water transparency can be measured directly as light attenuation. Dissolved and particulate material affect the rate at which light intensity attenuates with depth. Light intensity declines exponentially with depth allowing for the calculation of a vertical extinction coefficient ( $k$ ), or the rate of attenuation, and the depths at which there remains 10% and 1% of surface irradiance ( $Z_{10\%}$  and  $Z_{1\%}$ , respectively). These parameters are commonly measured for the wavelengths of light used for photosynthesis (between 400-700 nm, or photosynthetically active radiation; PAR). Note that  $k$  and  $Z$  are inversely related: as attenuation rate increases, the depths at which 10% or 1% surface irradiation remains decrease.

Light profiles were measured in Big and Little Twin on 20 July, 31 Aug, and 25 Sep and in Walker on 31 Aug and 25 Sep (Figure 6).

Light attenuation rate increased in Big Twin over the three sampling dates with  $k = 0.749$  on 20 July,  $k = 0.929$  on 31 Aug, and  $k = 1.15$  on 25 Sep.  $Z_{10\%}$  in Big Twin across these dates was 3.07 m, 2.48 m, and 2.00 m, respectively and  $Z_{1\%}$  was 6.14 m, 4.96 m, and 4.00 m, respectively.

Little Twin was more transparent than Big Twin during all sampling dates. Light attenuated faster with depth in Little Twin during the Aug and Sep samplings compared to the July sampling with  $k = 0.547$  on 20 July,  $k = 0.844$  on 31 Aug, and  $k = 0.835$  on 25 Sep.  $Z_{10\%}$  in Little Twin across these dates was 4.21 m, 2.73 m, 2.76 m, respectively, and  $Z_{1\%}$  was 8.42 m, 5.46 m, and 5.52 m, respectively.



**Figure 6: Light attenuation parameters measured in TWCWC lakes in 2021. Note orientation of the Y axes.**

Walker was the least transparent TWCWC lake during the Aug and Sep samplings with  $k = 1.95$  on 31 Aug and  $k = 1.71$  on 25 Sep.  $Z_{10\%}$  in Walker on these dates was 1.18 m and 1.35 m, respectively, and  $Z_{1\%}$  was 2.37 m and 2.70 m, respectively.

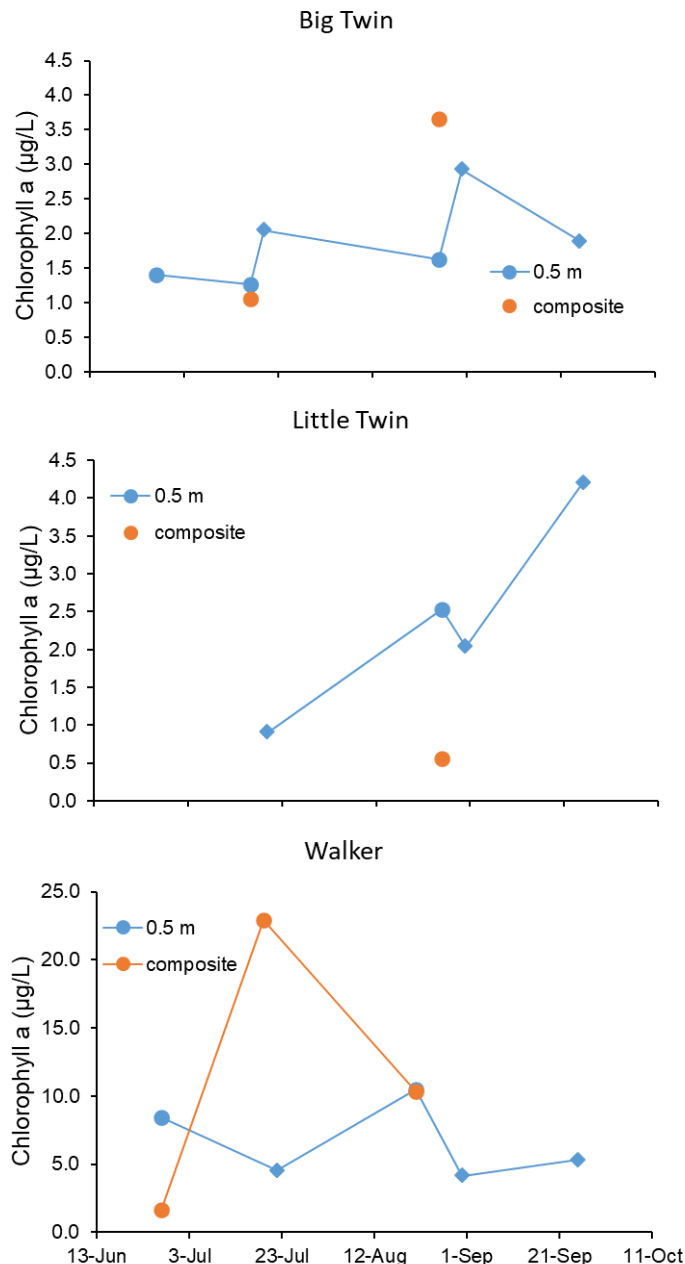
The decrease in transparency observed in late August and September in the Twin lakes was likely due to lingering effects of the late summer hurricanes.

#### IV. Chlorophyll Results

Chlorophyll *a* (chl<sub>a</sub>) is a pigment found in algal cells and is used as a proxy for algal abundance and lake productivity. PLEON measured chl<sub>a</sub> concentration in a sample collected from 0.5 m and in a composite sample collected by TWCWC. The PAWRRC project measured chl<sub>a</sub> concentration in duplicate samples collected from 0.5 m. Note that some samples are missing from the dataset.

Chl<sub>a</sub> concentration at 0.5 m in Big Twin ranged from 1.06 µg/L to 2.93 µg/L over the summer and early fall with peak concentration in late August (Figure 7). The composite sample collected on 26 August had more chl<sub>a</sub> than the surface sample, suggesting algae was concentrated in the middle-deep waters.

Chl<sub>a</sub> concentration at 0.5 m in Little Twin ranged from 0.91 µg/L to 4.21 µg/L over the summer and early fall, with algae abundance generally increasing over this time (Figure 7). Unlike Big Twin, the composite sample collected on 26 August from Little Twin had considerably less chl<sub>a</sub> than the surface sample, suggesting that algae were concentrated at the



**Figure 7: Chlorophyll concentration in TWCWC lakes in 2021. Circle and diamond symbols denote collections by TWCWC and the PAWRRC project, respectively. Note differences in scale of Y axes.**

surface during this collection. The depressed metalimnetic oxygen maximum observed on this date supports this suggestion.

Walker was the most productive of the three lakes in 2021 with chl<sub>a</sub> concentration at 0.5 m in Walker ranged from 4.14 µg/L to 10.5 µg/L (Figure 7). The chl<sub>a</sub> concentration of the composite samples were variable in comparison to the surface samples. On 22 July, the composite sample contained approximately 5x more chl<sub>a</sub> compared to the surface sample.

TSI can be calculated from chlorophyll *a* concentrations measured at 0.5 m according to the following equation<sup>4</sup>:

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

The TSI<sub>chlorophyll</sub> of Big Twin was 33.9, 32.9, 37.6, 35.4, 41.1, and 36.8 during the 27 June, 17 July, 20 July, 26 August, 31 August, and 25 September samplings, respectively, classifying Big Twin as oligotrophic on all dates except 31 August when it was mesotrophic (Table 5).

The TSI<sub>chlorophyll</sub> of Little Twin was 29.6, 39.7, 37.6, and 44.7 during the 20 July, 26 August, 31 August, and 25 September samplings, respectively, classifying Little Twin as oligotrophic on all sampling dates except 25 September when it was mesotrophic (Table 5).

The TSI<sub>chlorophyll</sub> of Walker was 51.6, 45.4, 53.6, 44.5, and 47.0 during the 27 June, 22 July, 21 August, 31 August, and 25 September samplings, respectively, classifying Walker as mesotrophic during the 22 July, 31 August, and 25 September samplings and as eutrophic during the 27 June and 21 August samplings (Table 5).

## V. Nutrient Results

### A. Total nitrogen

Total nitrogen (TN) concentration in samples collected from 0.5 m from Big Twin ranged from below detection to 0.51 mg/L in the summer of 2021 (Figure 8). Composite samples had more TN than surface samples (composite samples ranged from 0.57 mg/L to 0.88 mg/L), indicating greater nitrogen concentrations in deeper waters.

Total nitrogen (TN) concentration in samples collected from 0.5 m from Little Twin ranged from 0.11 mg/L to 0.73 mg/L in the summer of 2021 (Figure 8). Unlike Big Twin, composite samples from Little Twin had lower TN concentration during the June and July samplings, indicating lower TN concentration in the deeper waters compared to the surface. This trend was reversed during the August sampling.

Total nitrogen (TN) concentration in samples collected from 0.5 m from Walker ranged from 0.41 mg/L to 0.90 mg/L in the summer of 2021 (Figure 8). TN concentration in the



composite samples were below detection during the July and August sampling, indicating much lower TN concentration in the deeper waters.

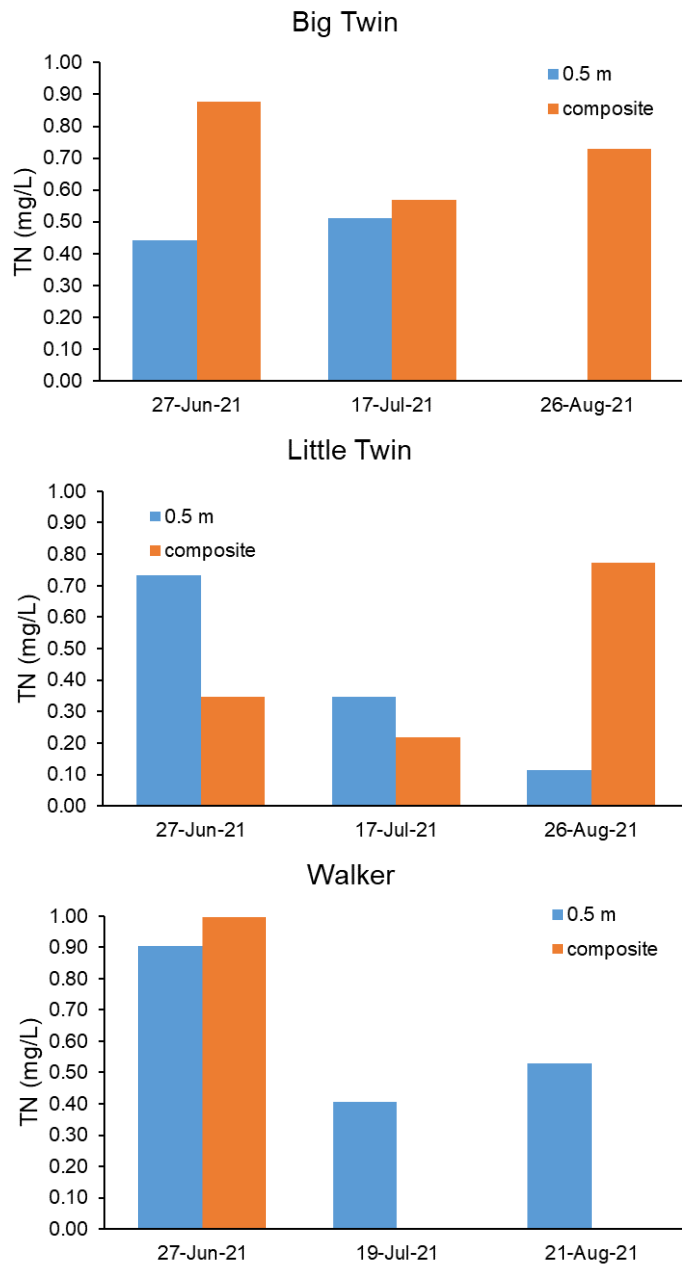
Nitrogen is an essential nutrient for algae and other aquatic life. Elevated concentrations of nitrogen can be a sign of eutrophication, or nutrient enrichment, of lakes. TN concentrations in TWCWC lakes were below the 3 mg/L threshold used by Penn State Extension to indicate nitrogen pollution<sup>1</sup>.

Drivers of differences between surface and composite samples are difficult to determine. Algae and bacteria take up nitrogen, so TN concentrations can be low where algae are congregated, often in surface or metalimnetic waters. However, lake mixing, precipitation, and other disturbances can also affect nutrient profiles.

### B. Total phosphorus

Total phosphorus (TP) concentration in samples collected from 0.5 m in Big Twin ranged from below detection to 9.43 µg/L, peaking during the August sampling (Figure 9). TP concentration in composite samples was consistently greater than surface samples, indicating greater TP concentration in deeper waters.

Total phosphorus (TP) concentration in samples collected from 0.5 m in Little Twin ranged from below detection to 16.13 µg/L, also peaking during the August sampling (Figure 9). Composite samples from Little Twin had greater TP concentrations during the June and July sampling, but not the August sampling.

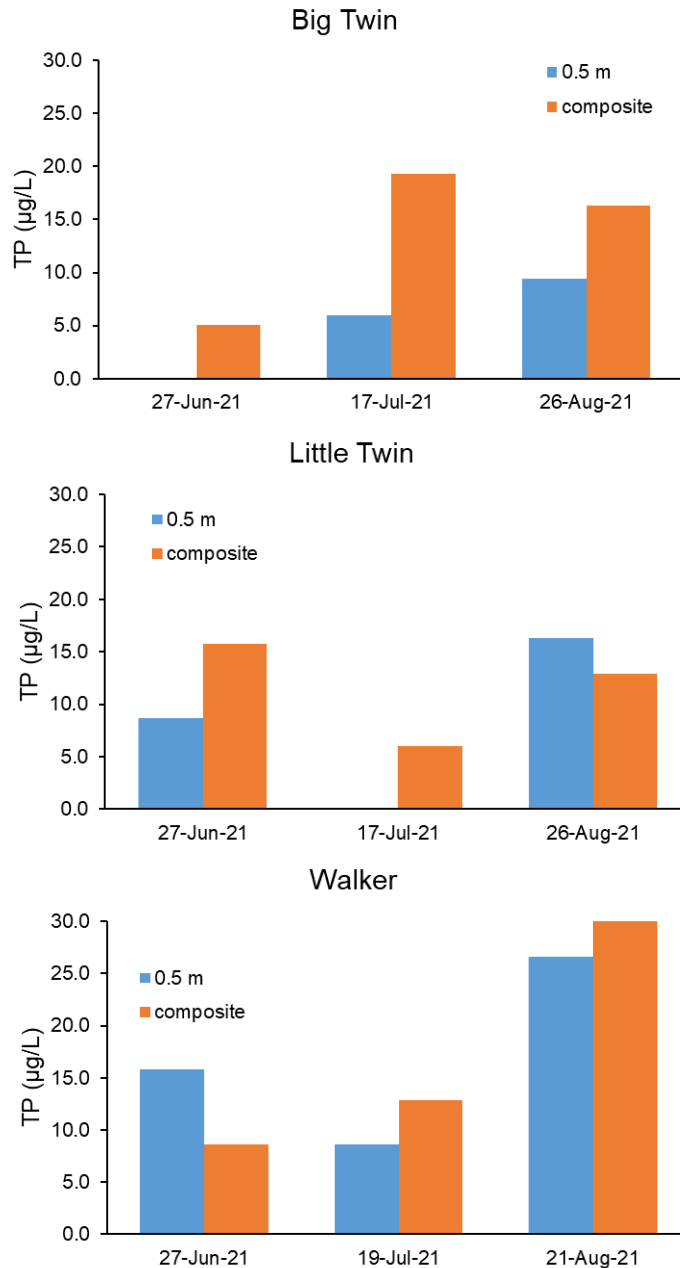


**Figure 8: TN concentration in TWCWC lakes in 2021. Note the different sampling dates among lakes. Missing bars were below detection.**

Total phosphorus (TP) concentration in samples from 0.5 m in Walker ranged from 8.63 µg/L to 26.6 µg/L, peaking during the August sampling like the other TWCWC lakes (Figure 9). TP concentration was greater in the composite samples compared to surface samples during the July and August sampling, but not the June sampling.

Like nitrogen, phosphorus is an essential nutrient for aquatic life and is often considered to be the primary nutrient limiting algal growth in lakes. Elevated concentrations of phosphorus can be a sign of eutrophication in lakes and can fuel algal blooms. TP concentration Walker exceeded the 25 µg/L threshold for nutrient pollution suggested by Penn State Extension<sup>1</sup> during the August sampling.

As with nitrogen, algae uptake of phosphorus can influence TP concentrations, particularly in the surface and metalimnetic waters. Phosphorus is also liberated from sediments under anoxic conditions, which can increase TP concentration in deep waters. Lake mixing and precipitation can affect nutrient profiles.



**Figure 9: TP concentration in TWCWC lakes in 2021. Note the different sampling dates among lakes. Missing bars were below detection.**

TSI can be calculated from TP concentration at 0.5 m as<sup>4</sup>:

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

TSI<sub>TP</sub> of Big Twin was undetermined during the June sampling (TP concentration below detection) and 29.6 and 36.0 during the July and August sampling, respectively. TSI<sub>TP</sub> classified Big Twin as oligotrophic during all three samplings (Table 5).

TSI<sub>TP</sub> of Little Twin was 34.8 during the June sampling, undetermined during July (TP concentration below detection), and 43.8 during the August sampling. TSI<sub>TP</sub> classified Little Twin as oligotrophic during June and July and as mesotrophic during August (Table 5).

TSI<sub>TP</sub> of Walker was 43.3, 34.8, and 50.8 during June, July, and August, respectively. TSI<sub>TP</sub> classified Walker as mesotrophic during June, oligotrophic during July, and eutrophic during August (Table 5).

### C. Dissolved organic carbon

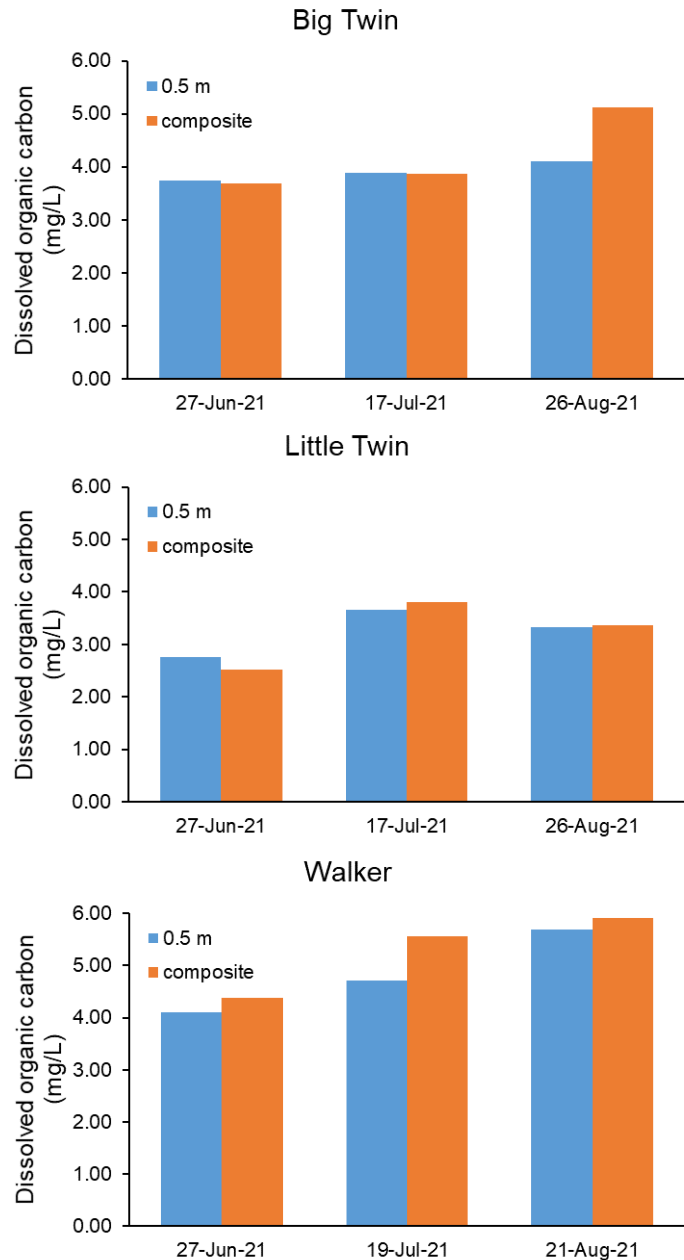
Dissolved organic carbon (DOC) concentration in surface samples was greatest in Walker, followed by Big Twin and Little Twin (Figure 10). Surface and composite samples generally had similar DOC concentrations. Exceptions were Walker's July sampling and Big Twin's August sampling. In both cases, composite samples had approximately 1 mg/L more DOC than surface samples.

DOC in lakes includes soluble organic compounds from runoff, byproducts of decomposition, and molecules synthesized within the water column<sup>5</sup>. DOC concentration is affected by the frequency and intensity of precipitation as well as watershed soil chemistry and structure.

## VI. Plankton Communities

### A. Zooplankton

Zooplankton are microscopic animals and key components of lake food webs. Zooplankton samples were collected from Big and Little Twin on 20 July 2021. Walker was not sampled for zooplankton.



**Figure 10: DOC concentration in TWCWC lakes in 2021. Note the different sampling dates among lakes.**

Zooplankton numbers in both lakes was dominated by rotifers, which made up 73% and 89% of zooplankton density in Big and Little Twin, respectively (Table 6). Rotifers eat detritus, bacteria, algae, and protozoans. Rotifers are small in size and made up 6% and 8% of zooplankton biomass in these lakes, respectively.

Cladocerans were the next most abundant group, making up 16% and 8% of zooplankton density in Big Twin and Little Twin, respectively. Copepods were slightly less abundant (11% and 8%, respectively). These groups are larger organisms (together making up 45% and 23% of biomass in these lakes, respectively) that eat algae and are important food for fish. The family Chaoboridae, while low in numbers, made up 49% and 69% of the zooplankton biomass in Big Twin and Little Twin, respectively. Chaoboridae, or phantom midges, are predatory insect larvae that eat zooplankton.

Community richness is the number of taxa present while diversity accounts for both the number and distribution of individuals among taxa. Average zooplankton richness in Big Twin and Little Twin was 12 and 16.5, respectively and average diversity (Shannon-Wiener Index) was 0.80 and 0.68, respectively.

**Table 6: Zooplankton communities in Big Twin and Little Twin on 20 July 2021.**

	Big Twin				Little Twin			
	Density		Biomass		Density		Biomass	
	(#/L)	(%)	(µg/L)	(%)	(#/L)	(%)	(µg/L)	(%)
<b>PROTOZOA</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
<b>ROTIFERA</b>	<b>252</b>	<b>73</b>	<b>20</b>	<b>6</b>	<b>283</b>	<b>89</b>	<b>26</b>	<b>8</b>
<i>Asplanchna</i>	0	0	0	0	7	2	7	2
<i>Conochilus</i>	38	11	2	<1	113	35	5	1
<i>Hexarthra</i>	0	0	0	0	1	<1	<1	<1
<i>Kellicottia</i>	7	2	<1	<1	3	1	<1	<1
<i>Keratella</i>	129	37	12	3	129	40	12	4
<i>Polyarthra</i>	71	20	6	2	27	8	2	1
<i>Trichocerca</i>	6	2	<1	<1	2	1	<1	<1
<b>COPEPODA</b>	<b>38</b>	<b>11</b>	<b>73</b>	<b>20</b>	<b>9</b>	<b>3</b>	<b>19</b>	<b>6</b>
Copepoda-Cyclopoida	24	7	37	10	5	1	8	2
Copepoda-Calanoidea	0	0	0	0	<1	<1	<1	<1
Other Copepoda-Nauplii	14	4	36	10	4	1	11	3
<b>CLADOCERA</b>	<b>57</b>	<b>16</b>	<b>91</b>	<b>25</b>	<b>26</b>	<b>8</b>	<b>55</b>	<b>17</b>
<i>Bosmina</i>	42	12	41	11	16	5	16	5
<i>Ceriodaphnia</i>	13	4	35	10	5	1	12	4
<i>Daphnia dubia</i>	0	0	0	0	2	<1	16	5
<i>Diaphanosoma</i>	0	0	0	0	2	<1	2	<1
<i>Holopedium</i>	2	1	16	4	1	<1	10	3
<b>OTHER ZOOPLANKTON</b>	<b>&lt;1</b>	<b>&lt;1</b>	<b>180</b>	<b>49</b>	<b>&lt;1</b>	<b>&lt;1</b>	<b>226</b>	<b>69</b>
Chaoboridae	<1	<1	180	49	<1	<1	226	69
<b>TOTAL</b>	<b>347</b>		<b>365</b>		<b>319</b>		<b>326</b>	

## B. Phytoplankton

Phytoplankton, or algae, are the base of planktonic food webs and help regulate oxygen dynamics in lakes. Phytoplankton were sampled from the Twin lakes on 20 July 2021.

Diatoms (or Bacillariophyta) were the numerically dominant group in Big Twin, making up 69% of phytoplankton density (Table 7). Chlorophyta, or the green algae, made up 17% of the phytoplankton density with other phytoplankton groups making up 11.5% combined. Importantly, Cyanophyta (cyanobacteria) were absent in Big Lake samples.

In contrast, Cyanophyta were the most abundant group in Little Twin, making up 69% of phytoplankton density (Table 7). The cyanobacteria community was composed of taxa of concern, including *Dolichospermum* and *Planktothrix*. Members of these genera are capable of producing several types of cyanotoxins that are harmful to humans and pets.

Chrysophyta, or golden algae made up 19% of phytoplankton density but 70% of the phytoplankton biomass. Chlorophyta, or green algae, made up <1% of phytoplankton density and <1% of phytoplankton biomass.

Average phytoplankton taxonomic richness in Big Twin was 11 and average diversity (measured using the Shannon-Wiener Index) was 0.58. Planktonic richness in Little Twin was 11.5 and diversity was 0.59.

**Table 7: Phytoplankton communities in Big Twin and Little Twin on 20 July 2021**

	Big Twin				Little Twin			
	Density		Biomass		Density		Biomass	
	(#/ml)	(%)	(µg/ml)	(%)	(#/ml)	(%)	(µg/ml)	(%)
<b>BACILLARIOPHYTA</b>	<b>377</b>	<b>69</b>	<b>302</b>	<b>37</b>	<b>246</b>	<b>9</b>	<b>160</b>	<b>7</b>
Araphid Pennate Diatoms	351	64	281	34	246	9	160	7
Biraphid Pennate Diatoms	26	5	20	2	0	0	0	0
<b>CHLOROPHYTA</b>	<b>91</b>	<b>17</b>	<b>105</b>	<b>13</b>	<b>23</b>	<b>&lt;1</b>	<b>7</b>	<b>&lt;1</b>
Flagellated Chlorophytes	49	9	5	<1	0	0	0	0
Coccoid/Colonial Chlorophytes	8	1	3	<1	16	<1	2	<1
Filamentous Chlorophytes	0	0	0	0	0	<1	0	0
Desmids	35	6	97	12	7	0	5	<1
<b>CHRYSOPHYTA</b>	<b>12</b>	<b>2</b>	<b>24</b>	<b>3</b>	<b>510</b>	<b>19</b>	<b>1,519</b>	<b>70</b>
Flagellated Classic Chrysophytes	8	1	23	3	506	19	1,519	70
Tribophytes/Eustigmatophytes	4	<1	<1	<1	3	<1	<1	<1
<b>CRYPTOPHYTA</b>	<b>13</b>	<b>2</b>	<b>20</b>	<b>2</b>	<b>15</b>	<b>&lt;1</b>	<b>13</b>	<b>&lt;1</b>
<b>CYANOPHYTA</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1,842</b>	<b>69</b>	<b>89</b>	<b>4</b>
Filamentous Nitrogen Fixers	0	0	0	0	372	14	74	3
Filamentous Non-Nitrogen Fixers	0	0	0	0	1,470	55	15	<1
<b>EUGLENOPHYTA</b>	<b>13</b>	<b>2</b>	<b>13</b>	<b>2</b>	<b>7</b>	<b>&lt;1</b>	<b>7</b>	<b>&lt;1</b>
<b>PYRRHOPHYTA</b>	<b>41</b>	<b>7.5</b>	<b>360</b>	<b>44</b>	<b>22</b>	<b>&lt;1</b>	<b>362</b>	<b>17</b>
<b>TOTAL</b>	<b>547</b>		<b>824</b>		<b>2,665</b>		<b>2,157</b>	

## VII. PTOX Cyanobacteria Screen

Cyanobacteria (sometimes called blue-green algae) are a common group of photosynthetic bacteria often classified as algae. Some cyanobacteria are capable of producing toxins that can be harmful to wildlife, pets, and humans. Cyanobacteria are the algae most commonly responsible for harmful algal blooms, or HABs, in freshwater ecosystems. Potentially toxigenic (PTOX) cyanobacteria genera can be identified using a microscope.

PLEON collected a sample from an area with a previously visible bloom along the shores of Big Twin Lake. The sample was collected on 2 June 2021 from wrist depth and was shipped to Greenwater Laboratories for microscopic analysis (Appendix I).

*Dolichospermum* sp. was found in the sample (Table 8). The recommended analyses for saxitoxin, anatoxin-a, and cylindrospermopsin concentration was declined by TWCWC.

**Table 8: Summary of PTOX screens and cyanotoxin analysis of samples collected from Big Lake on 2 June 2021. Screens were conducted by Greenwater Laboratories**

		Observed Taxa (count*; potential toxins**)	Greenwater recommendation	Results
2 June 2021	Sample A Big Lake Shoreline (wrist depth)	<i>Dolichospermum</i> c.f. <i>flos-aquae</i> . (10; MCs, CYL, ATX, STX)	Test for STX, CYL, ATX	Testing declined by TWCWC

\*Counts are filaments/ml. \*\*STX = saxitoxins, CYL = cylindrospermopsin, ATX = anatoxins, MCs = microcystins.

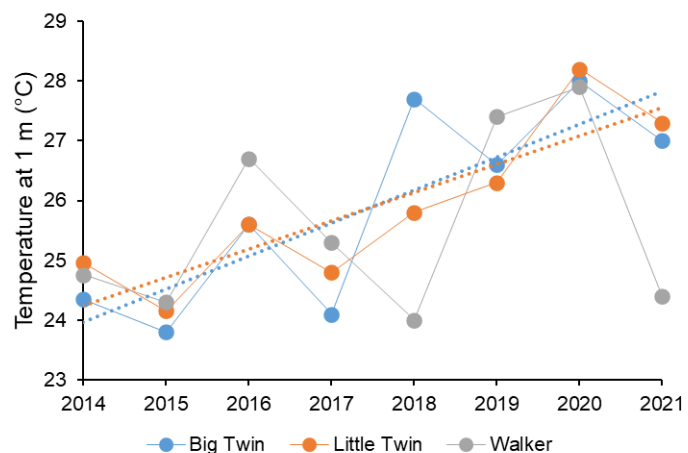
## VIII. Historical Context: Twin and Walker Lakes Over Time

### A. Description of historical dataset

PLEON began monitoring the Twin and Walker lakes in 2019. Data from 2014-2018 were provided by the TWCWC in the form of yearly “state of the lake” reports by FX Browne. In 2021, the Twin and Walker lakes were also a part of the PAWRRC project as previously described.

### B. Chemical profiles over time

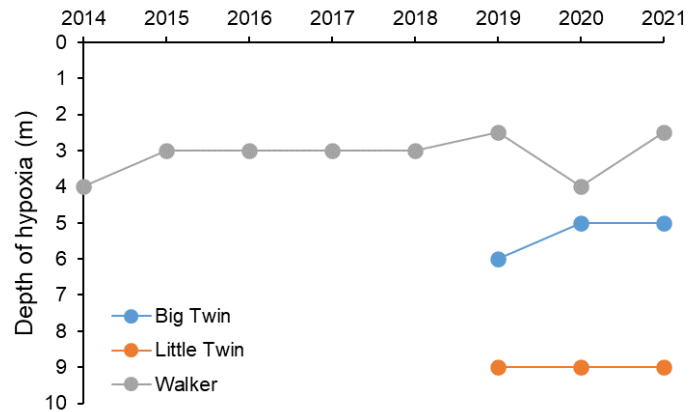
Chemical profiles in Big Twin and Little Twin are incomplete for much of the dataset as the TWCWC probe did not extend to



**Figure 11: Surface temperature in TWCWC lakes over time. Blue and orange dotted lines are linear regressions in Big Twin ( $temp = 0.55 \cdot year - 1082.53$ ) and Little Twin ( $temp = 0.47 \cdot year - 927.139$ ).**

the bottom of these lakes until 2021. Prior to 2021, complete depth profiles exist for these lakes in July of 2019 and 2020. All Walker Lake profiles are complete.

The three TWCWC lakes were generally stratified in the summer months (June, July, August) from 2014-2021 (Appendix V). Surface temperature in Big Twin and Little Twin, while variable, has significantly increased over this time period (linear regression,  $p \leq 0.02$ ,  $r^2 \geq 0.65$ , comparing temperature at 1 m during July sampling; Figure 11). Surface temperature in Walker Lake was more variable over this time period and there was no statistically significant increase.



**Figure 12: Depth of oxygen depletion (< 2 mg/L dissolved oxygen concentration) in TWCWC lakes since 2014.**

The TWCWC lakes were generally deplete of oxygen in the hypolimnion (Appendix V). Note that data for the Twin Lakes does not include hypolimnetic oxygen until 2019. Since 2019, the depth at which DO concentrations was less than 2 mg/L (the threshold for oxygen depletion) was deepest in Little Twin and most shallow in Walker (measured in July; Figure 12). This depth has remained fairly consistent in Walker Lake since 2014. Note that these data are missing for the Twin Lakes before 2019. Metalimnetic oxygen maxima were common in Little Twin during the summer months and occurred occasionally in Big Twin as well.

Conductivity in Little Twin was consistently greater than that of Big Twin and Walker across the dataset, with an average conductivity of 155  $\mu\text{S}/\text{cm}$  compared to 87  $\mu\text{S}/\text{cm}$  and 90  $\mu\text{S}/\text{cm}$  in the other lakes, respectively (averages include all depths in June, July, and August of all years; Appendix V). Conductivity measured in all lakes during June 2015 was high relative to other years. This may have been due to increased runoff or evaporation.

pH in Walker Lake was generally lower than that of Big Twin and Little Twin across the dataset, with an average pH of 6.73 compared to 7.08 and 7.33 in the other lakes, respectively (averages include all depths in June, July, and August of all years; Appendix V). However, this may be due to missing hypolimnetic data in the Twin lakes; pH tended to be lower in the deep water. pH in June of 2015 tended to be lower than other years in all three lakes, corresponding with the increased conductivity observed at this time. Increased precipitation could have caused lower pH (and more runoff) as rainwater tends to have lower pH compared to lake water. Similarly, the concentrating effect of evaporation would have increased conductivity and decreased pH.

TDS concentration generally followed the same pattern as conductivity in all three lakes over time (Appendix V). Little Twin had greatest TDS concentration across dataset (average of 105.2 mg/L), followed by Walker (average of 63.3 mg/L) and Big Twin (average of 59.0 mg/L).

### C. Water transparency over time

Secchi depth in the TWCWC lakes has been measured since 2014, allowing for a more robust temporal analysis of water clarity in these lakes. Little Twin was the clearest lake with an average summer Secchi depth of 3.7 m, followed by Big Twin with an average summer Secchi depth of 2.7 m, and Walker with an average Secchi depth of 1.4 m (averages include all readings in June, July, and August from 2014-2021; Figure 13). Transparency within lakes was relatively consistent over the 8 years with no clear trends over time to date.

Summer light attenuation parameters have been measured in TWCWC lakes yearly since 2019 (twice in 2021). Over this time, Little Twin was the most transparent (with  $k$  ranging from 0.4-0.7), followed by Big Twin ( $k$  ranging from 0.8-1.0) and Walker ( $k$  ranging from 1.4-1.8; Figure 14). Transparency in Little Twin and Walker decreased from 2020 to 2022.

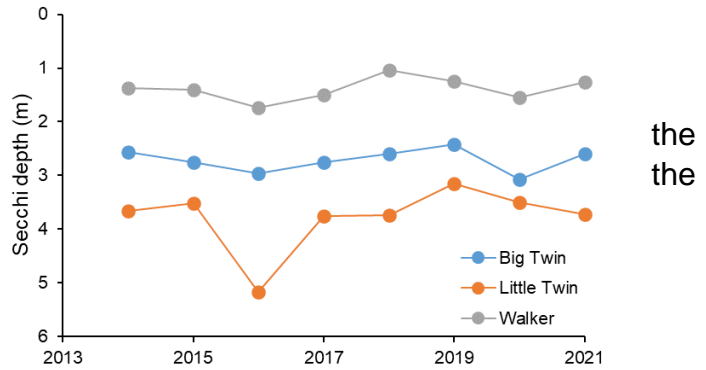


Figure 13: Average summer Secchi depth since 2014.

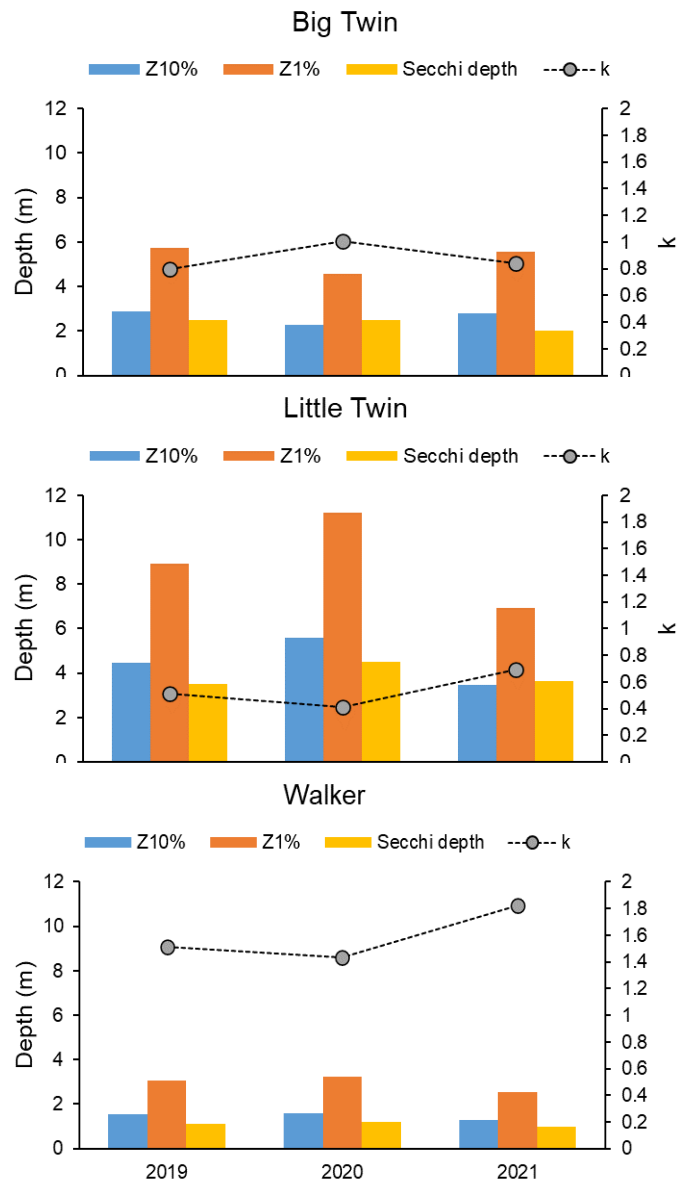
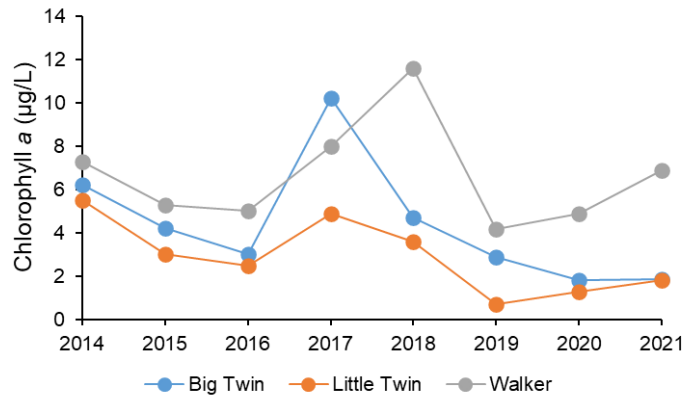


Figure 14: Water transparency in TWCWC lakes from July 2019-2021. Note orientation of Y axes.



#### D. Chlorophyll a over time

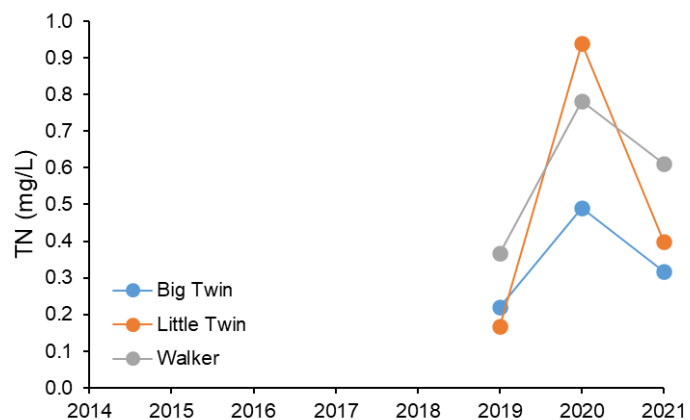
Chla concentration has been measured in TWCWC lakes since 2014. Over this time, average summer (June, July, August) chla concentration at 0.5 m has ranged from 1.82 µg/L to 10.2 µg/L in Big Twin, from 0.71 µg/L to 5.5 µg/L in Little Twin, and from 4.19 µg/L to 11.6 µg/L in Walker (Figure 15). Chla concentrations have been on the low end of those ranges since 2019 in all three lakes. Chla concentration has been increasing since 2019 in Little Twin and, more dramatically, in Walker.



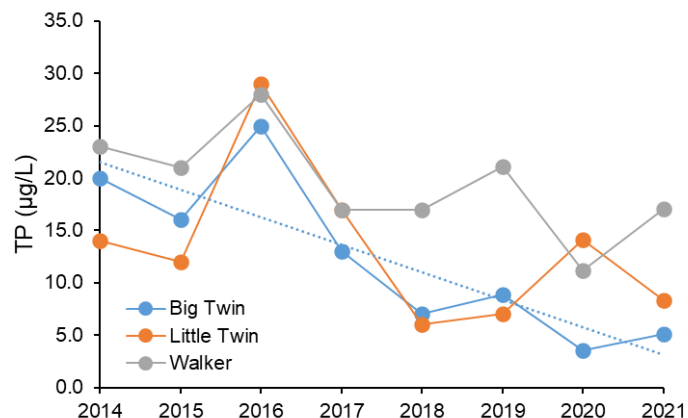
**Figure 15: Average summer chlorophyll concentration measured from samples collected from 0.5 m in TWCWC lakes since 2014.**

#### E. Nutrients over time

TN concentration has been measured in TWCWC lakes since 2019. Average summer (June, July, August) TN concentration measured in samples collected from 0.5 m ranged from 0.22 mg/L to 0.49 mg/L in Big Twin, from 0.17 mg/L to 0.94 mg/L in Little Twin, and from 0.37 mg/L to 0.78 mg/L in Walker (Figure 16). The greatest TN concentration occurred in 2020 in all three lakes. There is not enough data to assess meaningful temporal trends in TN concentration at this time.



TP concentration has been measured in TWCWC lakes since 2014. Average summer (June, July, August) TP measured in samples collected from 0.5 m ranged from 3.54 µg/L to 25.0 µg/L in Big Twin, from 6.0 µg/L to 29.0 µg/L in Little Twin, and from 11.2 µg/L to 28.0 µg/L in



**Figure 16: Average summer TN (top) and TP (bottom) concentration measured from 0.5 m over time in TWCWC lakes. Dotted line in bottom panel is a linear regression of TP concentration in Big Twin over time (TP concentration =  $-2.63 * \text{year} + 5310.9$ ).**

Walker (Figure 16). Summer TP concentration has been generally declining over the 8 period in all lakes. This decline is statistically significant in Big Twin (linear regression,  $r^2 = 0.72$ ,  $p = 0.008$ ), but not Little Twin or Walker.

DOC concentration was quantified in August of 2020 and in June, July, and August 2021 in all three lakes (data not shown). There is as yet insufficient data to determine if the TWCWC lakes are “browning” or experiencing an increase in DOC concentration over time as are some other lakes in the Pocono region<sup>6</sup>.

#### F. Trophic status over time

Big Twin and Little Twin have been generally classified as meso-oligotrophic lakes since 2014, depending on the metric used to calculate TSI. Walker has been generally classified as meso-eutrophic over this time period, again depending on TSI metric.  $TSI_{Secchi}$  has been relatively stable over the 8-year dataset in all three lakes.  $TSI_{chlorophyll}$  and  $TSI_{TP}$  have declined in Big Twin since 2018 but have been more variable in the other two TWCWC lakes (Figure 17).

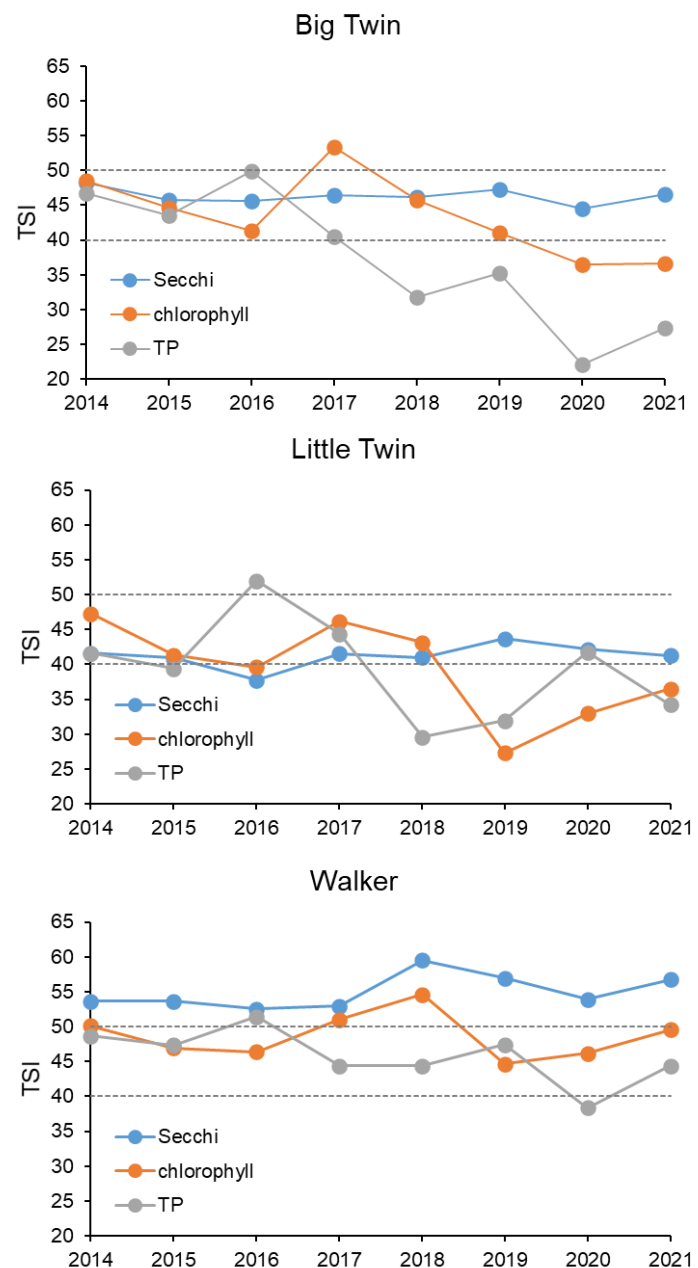
#### G. Zooplankton over time

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

Zooplankton were less abundant in 2021 compared to 2020 by ~30% in Big Twin and by ~7% in Little Twin (Figure 18).

Zooplankton density in both lakes was greater in 2021 than in 2020, by ~3x in Big Twin and by more than 2x in Little Twin.

Zooplankton community composition in Big Twin shifted in 2021 compared to 2020 with an

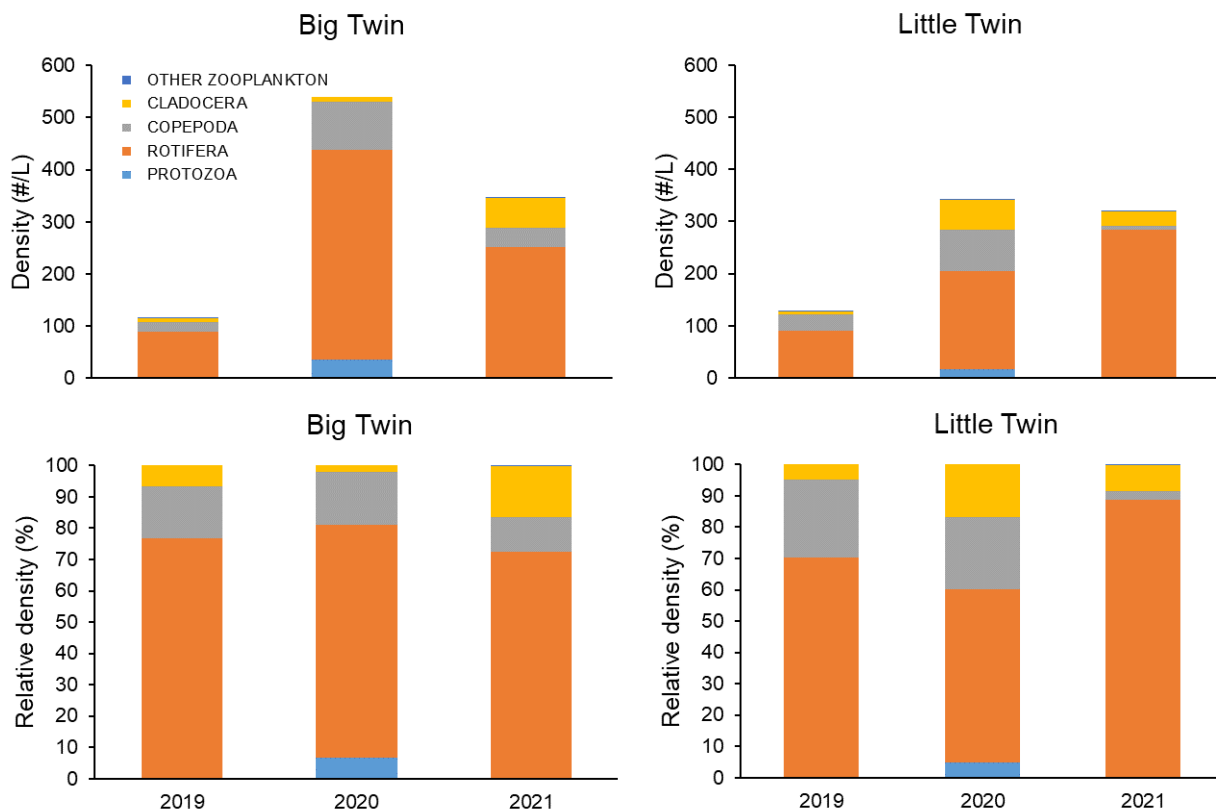


**Figure 17: Trophic status of TWCWC lakes since 2014. Dashed lines indicate oligotrophy (<40), mesotrophy (40-50), and eutrophy (>50).**

increase in cladoceran relative abundance and a decrease in protozoan and copepod relative abundance. In contrast, cladocerans made up a smaller proportion of zooplankton density in 2021 in Little Twin compared to 2020, along with copepods and protozoans. Rotifers increased in relative abundance in Little Twin from 2020 to 2021.

The zooplankton community in Big Twin had similar richness over the three year dataset (Table 9). Zooplankton diversity in Big Twin was somewhat variable across the three years. Average zooplankton length increased in 2021 compared to 2020 (by 0.03 mm) and 2019 (by 0.02 mm), likely reflecting the increase in cladocerans, which are larger than rotifers. The average length of individuals is an important metric for zooplankton communities as large zooplankton are efficient “algae eaters” and can regulate algae communities.

Little Twin zooplankton community had similar richness in 2021 as in 2022 but was less diverse, suggesting a less even distribution of individuals among similar number of groups (Table 9). Average zooplankton length was shorter by 0.12 mm in 2021 compared to 2020, likely reflecting the shift from the larger cladocerans and copepods to smaller rotifers.



**Figure 18: Zooplankton density (top panels) and relative density (bottom panels) in Big and Little Twin since 2019.**

**Table 9: Plankton richness, diversity, and average length in Big and Little Twin since 2019.**

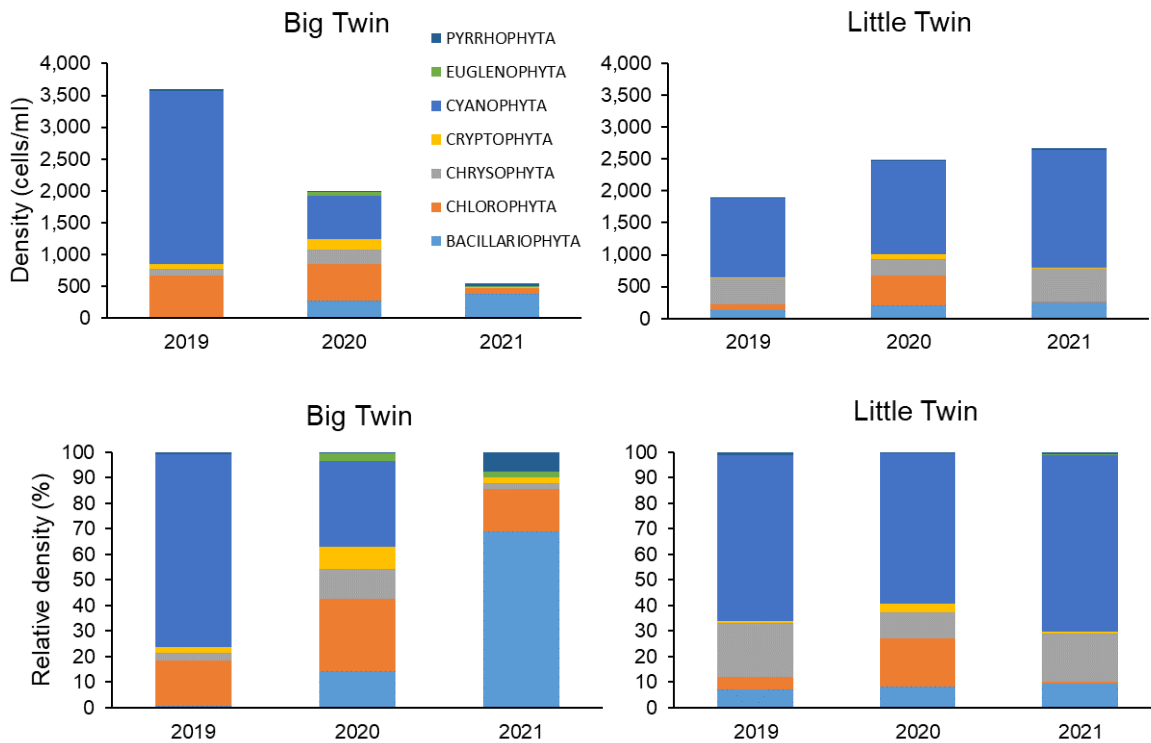
	2019	Big Twin 2020	2021	2019	Little Twin 2020	2021
<b>Zooplankton</b>						
Richness	12	11.5	12	13.5	16	16.5
Diversity*	0.72	0.84	0.75	0.72	0.95	0.68
Mean Length	0.17	0.16	0.19	0.19	0.27	0.15
<b>Phytoplankton</b>						
Richness	20	17	11	12	17	12
Diversity*	0.64	1.06	0.58	0.59	0.94	0.59

\*Shannon-Wiener diversity index

#### H. Phytoplankton over time

Phytoplankton density decreased substantially from 2019 to 2021 in Big Twin with 2021 density ~33% that of 2020 (Figure 19). This decrease may be due to the increase in cladocerans in Big Twin, at least from 2020 to 2021 as cladocerans are voracious algae-eaters. Phytoplankton composition also shifted over the three-year dataset, most notably with a decrease in Cyanophyta (from 75% relative abundance in 2019 to 0% in 2021) and an increase in Bacillariophyta (from <1% in 2019 to 69% in 2021) over time.

In contrast, phytoplankton density in Little Twin increased over the three-year dataset with phytoplankton density in 2021 roughly 30% greater than in 2019 (Figure 19). Interestingly, there was no substantial increase in cladocerans in Little Twin over this



**Figure 19: Phytoplankton density (top panels) and relative density (bottom panels) in Big and Little Twin since 2019.**

time period and the density of copepods, another group of algae-eating zooplankton, actually decreased in relative abundance. Shifts in phytoplankton composition over time were also different in Little Twin compared to Big Twin. Relative abundance of Cyanophyta was greatest in 2021 (69%) compared to 2019 and 2020 (65% and 59%, respectively) and Chlorophyta relative abundance decreased from 18% in 2020 to <1% in 2021.

Phytoplankton richness and diversity in both Twin lakes decreased from 2020 to 2021. These metrics were similar in 2021 to 2019 in Little Twin but were still depressed in Big Twin (Table 9).

#### I. Cyanobacteria and cyanotoxins over time

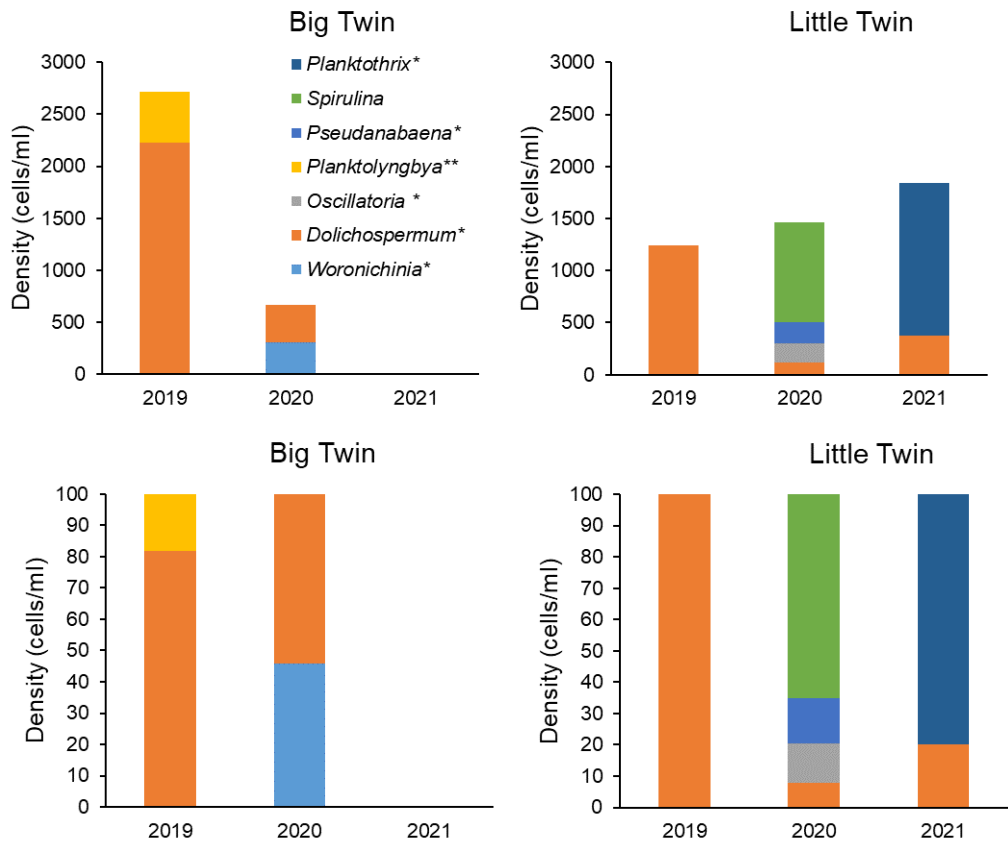
Samples from all three lakes were screened for potentially toxigenic (PTOX) cyanobacteria in 2019 and 2020, as part of the Pocono Lakes HABs Survey (Lauren Knose, Miami University), and by the PA Harmful Algae Bloom Task Force monitoring program, respectively. A single sample from Big Twin Lake was screened through PLEON in 2021. All PTOX screens and cyanotoxin analysis (2020 and 2021 only) were conducted by Greenwater Laboratories. As previously described, in-depth phytoplankton community analysis has been conducted on samples collected from the Twin Lakes since 2019.

Cyanobacteria communities have been variable over the three-year dataset in all three TWCWC lakes, as reflected in the comprehensive community analyses (Twin lakes only) and the PTOX screens.

Cyanobacteria made up 76%, 34%, and 0% of the phytoplankton communities collected from the center of Big Twin in 2019, 2020, and 2021, respectively (Figure 20). 100% of the cyanobacteria observed in 2019 and 2020 were genera thought to be capable of producing cyanotoxins. Two visible cyanobacteria blooms have been sampled along the shores of Big Twin Lake in the past 3 years, both containing *Dolichospermum* (Table 10). Note that the bloom sampled in 2021 occurred in June along the shoreline while the analysis of the phytoplankton community collected at center lake in July contained no cyanobacteria.

Cyanobacteria made up 65%, 59%, and 69% of the phytoplankton communities collected from the center of Little Twin in 2019, 2020, and 2021, respectively (Figure 20). Cyanobacteria composition varied among the three years with *Dolichospermum*, *Spirulina*, and *Woronichinia* the dominant genera in 2019, 2020, and 2021, respectively. PLEON (and collaborations) has not observed visible cyanobacteria blooms in Little Twin, but PTOX screens (Table 10) and detailed analyses of phytoplankton communities in this lake contained potentially toxigenic taxa in 2019 and 2020.

PLEON (and collaborations) has not observed visible cyanobacteria blooms in Walker, but PTOX screens have contained potentially toxigenic taxa in 2019 and 2020 (Table 10).



**Figure 20: Cyanobacteria density (top) and relative abundance (bottom) in Big Twin and Little Twin in 2019-2021**

**Table 10: Results of PTOX screens of TWCWC lakes.**

	Program	Location	Observations	PTOX genera	Toxins*
Big Twin	17 Jul 2019 Knose Survey	Center (0.5 m)	No bloom visible	<i>Dolichospermum</i>	Testing not recommended
	24 Aug 2020 PA HABs Task Force	Deiner dock (wrist)	Visible bloom along the shoreline day of collection	<i>Dolichospermum</i>	<MDL
	2 Jun 2021 PLEON	East dock (wrist)	Shoreline bloom visible days before.	<i>Dolichospermum</i>	Testing declined
Little Twin	24 July 2019 Knose Survey	Center (0.5 m), Dock (wrist)	No bloom visible	<i>Dolichospermum</i>	Testing not recommended
	24 Aug 2020 PA HABs Task Force	Dock (wrist)	No bloom visible	<i>Dolichospermum</i> <i>Aphanizomenon/Chrysochlorum</i>	<MDL
Walker	15 July 2019 Knose Survey	Center (0.5 m) Dock (wrist)	No bloom visible	<i>Aphanizomenon</i>	Testing not recommended
	24 Aug 2020 PA HABs Survey	Dock (wrist)	No bloom visible	<i>Chrysochlorum</i>	<MDL

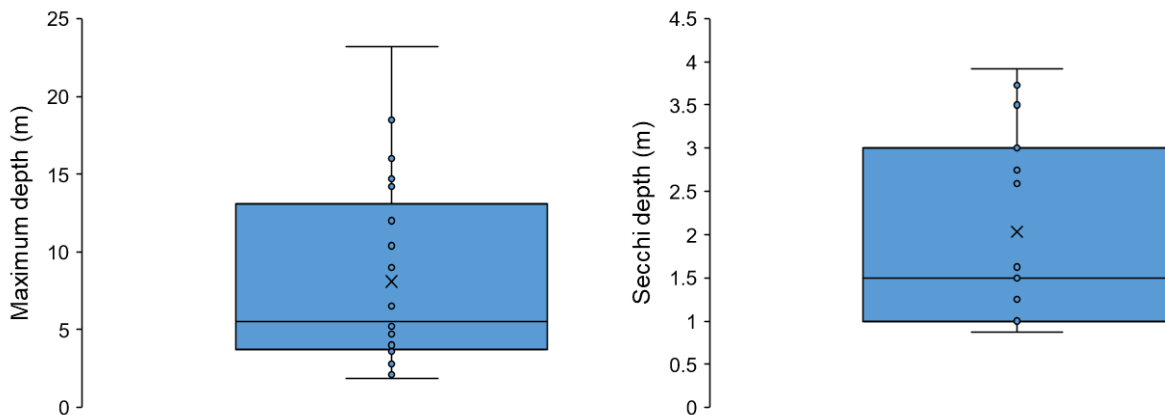
\*MDL=minimum detection limit

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

### A. Description of PLEON Lakes

The PLEON dataset consists of 21 lakes in Pike, Wayne, and Monroe Counties. Lakes range from ~29,000-1,130,000 m<sup>2</sup> (mean of ~363,000 m<sup>2</sup>) in surface area, ~900-7,800 m (mean of ~3,140 m) in shoreline and ~2-23 m (mean of 8 m) in depth (Figure 21). 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 shallower than the PLEON average.

The Secchi depth of the 15 PLEON lakes monitored in the summer of 2021 ranged from 0.88 m to 3.92 m with an average of 2.03 m (Figure 21). The 2021 summer Secchi depths (average of all readings in June, July, and August) of Big Twin and Little Twin lakes were deeper than the PLEON average while the 2021 summer Secchi depth of Walker was below the PLEON average. 5 of the PLEON lakes sampled in 2021 were of similar depth to the Twin Lakes. Big Twin's average summer Secchi depth was more shallow than the average of these lakes while Little Twin's was deeper. Walker's average summer Secchi depth was more shallow than the average of the 6 PLEON lakes of similar depth.



**Figure 21: Maximum depth of all PLEON lakes monitored to date (left) and average summer Secchi depth of PLEON lakes monitored in 2021 (right). Lines within boxes are median values and X symbols are means. Note the differences in scale between the panels.**

### B. Lake productivity

Lake productivity, as measured by chl<sub>a</sub> concentration at 0.5 m depth, was assessed in 16 PLEON lakes during the summer months (June, July, August) in 2021. Chl<sub>a</sub> concentration in these lakes ranged from 0.78 µg/L to 19.36 µg/L with an average of 6.84 µg/L (Figure 22). Average summer chl<sub>a</sub> concentration in Big Twin and Little Twin was below the PLEON average (1.97 µg/L and 1.83 µg/L, respectively) while chl<sub>a</sub> concentration in Walker (6.90 µg/L) was just above the PLEON average.

### C. PTOX Cyanobacteria

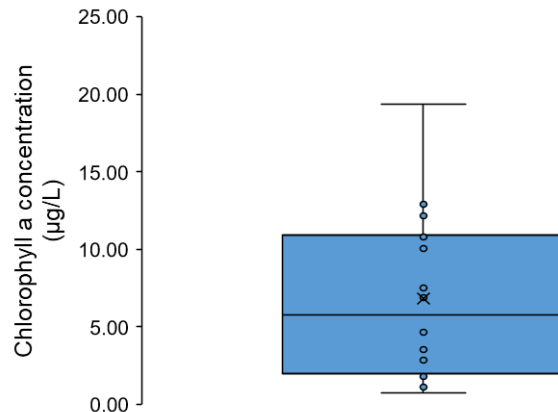
Since 2017, PLEON has collected 163 samples for PTOX screening as a part of its formal monitoring program. These samples were collected from 15 lakes during months ranging from May through September. This count includes samples collected from the same lake on the same day, but from different locations within the lake. Samples include collections from 0.5 m, surface grabs, and composite samples and include pelagic, shore and near-shore environments. All samples were screened by Greenwater Laboratories.

Ten (possibly 11, some specimens are difficult to identify) PTOX cyanobacteria genera have been found in PLEON samples to date (Figure 23). The most commonly found genera are *Dolichospermum*, followed by *Aphanizomenon* (or *Aphanizomenon*-like).

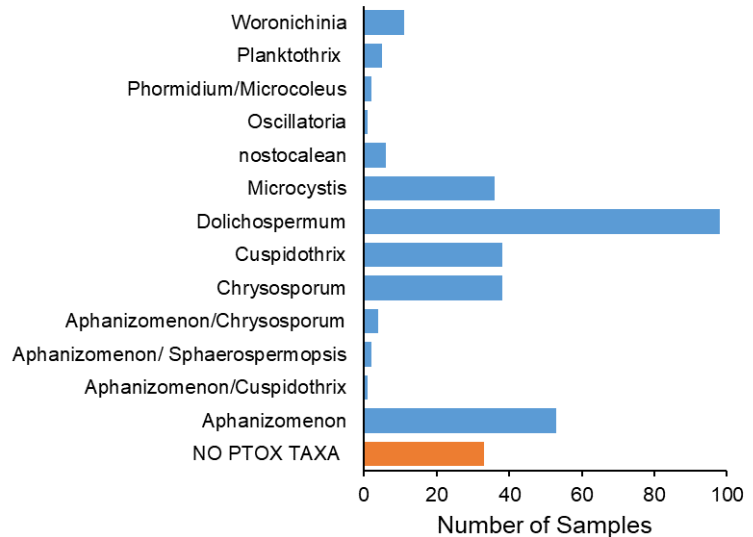
*Chrysochlorum*, *Woronichinia*, and *Microcystis* were also common. 33 of the samples (or 20%) did not have PTOX taxa present. Three lakes within the dataset have been consistently free of PTOX taxa, but note that these lakes were among the lakes sampled the least frequently.

*Dolichospermum*, *Aphanizomenon*, and *Chrysochlorum* species are commonly found in PTOX samples from TWCWC lakes. *Woronichinia*, *Planktothrix*, and *Oscillatoria* species have been found in phytoplankton community analysis of Big Twin and Little Twin (conducted by Water Resources), along with species of *Pseudanabaena*, *Spirulina*, and *Planktolyngbya*, genera not found in the PLEON PTOX database to date.

Microcystin/nodularins and cylindrospermopsin have been detected in PLEON lakes (Table 11). Microcystin/nodularins are hepatotoxins (affecting liver cells) and



**Figure 22: Average summer chlorophyll a concentration of PLEON lakes monitored in 2021. Line within the box is the median and the X symbol is the mean.**



**Figure 23: PTOX cyanobacteria genera found in 163 samples collected from PLEON lakes in 2017-2021.**



cylindrospermopsin is a hepatotoxin and a nephrotoxin (affecting kidney cells)<sup>7</sup>. The US Environmental Protection Agency recommends microcystin and cylindrospermopsin magnitude thresholds of 8 µg/L (or ng/mL) and 15 µg/L in recreational waters<sup>8</sup>. Pennsylvania does not have recommended thresholds at this time.

To date, cyanotoxins have not been detected in TWCWC lakes. Note that TWCWC declined the cyanotoxin testing recommended by Greenwater Laboratories in 2021.

**Table 11: Summary of PTOX screens tested for toxin concentration across PLEON lakes (2017-21)**

Toxin	# samples recommended for testing	# samples tested	# samples with detectable concentrations	Mean concentration (ng/mL)	Range (ng/mL)
microcystins/nodularins	62	46	20	9.4	0.16-129
cylindrospermopsin	41	25	1	0.07	-
anatoxin-a	40	27	0	-	-
saxitoxin	39	26	0	-	-
homoanatoxin-a	1	1	0	-	-

## X. 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:

### 2. Cyanobacteria blooms occur in Big Twin Lake and there is potential for them to occur in Little Twin and Walker.

Periodic screening for potentially toxigenic (PTOX) cyanobacteria has detected several PTOX cyanobacteria genera in all three TWCWC lakes, including *Dolichospermum*, *Aphanizonmenon*, and *Chrysoosporum*. PTOX cyanobacteria counts were high enough to prompt toxin analysis according to the PA DEP HABs Task Force protocols in August of 2020 in all 3 lakes. It is important to note that no bloom while there was noticeable algae on the surface of Big Twin, no visible bloom was present in Little Twin or Walker when these samples were taken. PTOX cyanobacteria counts from a sample collected from Big Twin in June of 2021 also prompted Greenwater Laboratories to recommend cyanotoxin testing.

Phytoplankton community analysis since 2019 in Big Twin and Little Twin also show the potential for cyanobacteria blooms in these lakes. While variable over time, several potentially toxigenic genera have been found in these lakes.

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](#). TWCWC may want to consider a HABs monitoring and response plan in 2022.

## **2. Changes in productivity and phosphorus in Little Twin should be monitored.**

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 seemed to stabilize in 2021. Emphasis should be placed on monitoring these parameters in Little Twin moving forward.

## **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<sup>9</sup>. 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<sup>6</sup>. 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.

# Report of 2020 PLEON Sampling: Twin and Walker Lakes

## APPENDICES

### APPENDIX I: Description of Field Sampling Methods

#### A. Physical Profiles

Temperature, dissolved oxygen, conductivity, and pH were measured using a handheld YSI Professional Plus multiparameter instrument fitted with a polarographic dissolved oxygen probe and a pro series pH probe. Probes were calibrated in early June 2021 and periodically through the summer. Probes were lowered through the water column starting at the surface (probes just under water, “0 m”). Readings were recorded in the field every 0.5-1 m.

Secchi depth was taken from the shady side of the boat using a Secchi Disk standard to freshwater sampling.

Light profiles were taken by lowering the sensor through the water column suspended off the side of the boat to avoid boat-shadow using a LiCOR spherical quantum sensor (model LI-193).

#### B. Chlorophyll

Chlorophyll a pigment was extracted from phytoplankton using the method developed by Robert Moeller and currently used by the Williamson Lab. Water samples were collected from the epilimnion, metalimnion (when appropriate), and hypolimnion (determined by temperature profile collected on the same day) using a Van Dorn bottle. Two replicate samples were collected from each depth. Samples were kept cold until filtered. For each replicate, a known volume was filtered onto a glass fiber filter with nominal pore size of 0.7  $\mu\text{m}$  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  $-20^{\circ}\text{C}$  with a 2-minute heating step ( $60^{\circ}\text{C}$ ) 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 replicate water samples were collected using a Van Dorn horizontal water sampler from the epilimnion, metalimnion, and hypolimnion. Water samples were collected in acid washed bottles and kept cold until return to the lab. A 60 ml subsample of each replicate was frozen at  $-20^{\circ}\text{C}$  until analysis for total nitrogen (TN) and total phosphorus (TP) at Lacawac Sanctuary.

Total nutrient samples were digested using an alkaline persulfate oxidizing reagent and heating at  $80^{\circ}\text{C}$  for 16-24 hours. This process simultaneously converts ammonium,

inorganic nitrogen (excluding N<sub>2</sub>), and organic nitrogen to nitrate (NO<sub>3</sub><sup>-</sup>) and inorganic and organic phosphorus to orthophosphate (PO<sub>4</sub><sup>-3</sup>).

NO<sub>3</sub>-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<sub>3</sub>-N concentration from a standard curve.

PO<sub>4</sub>-P concentration of the digested samples was quantified using the ascorbic acid colorimetric method with the absorbance measured at 650 nm used to calculate PO<sub>4</sub>-P concentration using a standard curve.

#### D. Dissolved organic carbon (DOC)

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

#### E. PTOX screening and cyanotoxin analysis

PLEON sends PTOX samples to GreenWater Laboratories for PTOX screening. Samples are kept cold in the field and sent to GreenWater Laboratories within 30 hours. GreenWater Labs provides the following description of the screening process:

“A one mL aliquot of each sample was prepared using a Sedgewick Rafter cell. The samples were scanned at 100X for the presence of potentially toxicogenic (PTOX) cyanobacteria using a Nikon Eclipse TE200 inverted microscope equipped with phase contrast optics. Higher magnification was used as necessary for identification and micrographs.”

Cyanotoxins were analyzed by Greenwater Laboratories using Enzyme-Linked Immunosorbent Assay (ELISA; microcystin-nodularins and saxitoxins) or Liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS; anatoxins and cylindrospermopsin) according to laboratory-specific protocols.

#### F. Plankton community analysis

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

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

Zooplankton samples were concentrated to at least 10,000x the original sample. Concentrated samples were homogenized and subsamples were counted using a

Sedgewick-Rafter counting chamber and bright-field microscopy. Biomass was determined using group-specific calculations.

## Appendix II: Literature Cited

1. 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>
2. Fondriest Environmental, Inc. "pH of Water." Fundamentals of Environmental Measurements. 19 Nov. 2013. Web. <https://www.fondriest.com/environmental-measurements/parameters/water-quality/ph/>.
3. Lehigh Environmental Initiative. "Total Dissolved Solids". Accessed 8 April 2022. Web. <https://ei.lehigh.edu/envirosci/watershed/wq/wqbackground/tdsbg.html>
4. Carlson, R. E. 1977. A trophic state index for lakes. *Limnology and Oceanography* 22(2): 361-369.
5. Fondriest Environmental, Inc. "Chromophoric Dissolved Organic Matter." Fundamentals of Environmental Measurements. 1 Aug. 2017. Web. < <https://www.fondriest.com/environmental-measurements/parameters/water-quality/chromophoric-dissolved-organic-matter/>.
6. Williamson, C. E. et al. 2015. Ecological consequences of long-term browning in lakes. *Scientific Reports* 5:18666 DOI: 10.1038/srep18666
7. GreenWater Laboratories. "What Are Algal Toxins?" Accessed 31 March 2022. Web. <https://www.greenwaterlab.com/what-are-algal-toxins/>
8. US EPA. Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin. Retrieved on 10 March 2020, <https://www.epa.gov/sites/production/files/2019-05/documents/hh-rec-criteria-habs-factsheet-2019.pdf>
9. 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

## Appendix III: Glossary

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

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

**Conductivity:** the ability of a solution to conduct electricity (also called specific conductance). Dissolved materials increase the conductivity of water so this

variable can indicate the amount of dissolved solids. Sea water, for example, has a conductivity of 50,000  $\mu\text{S}/\text{cm}$ .

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

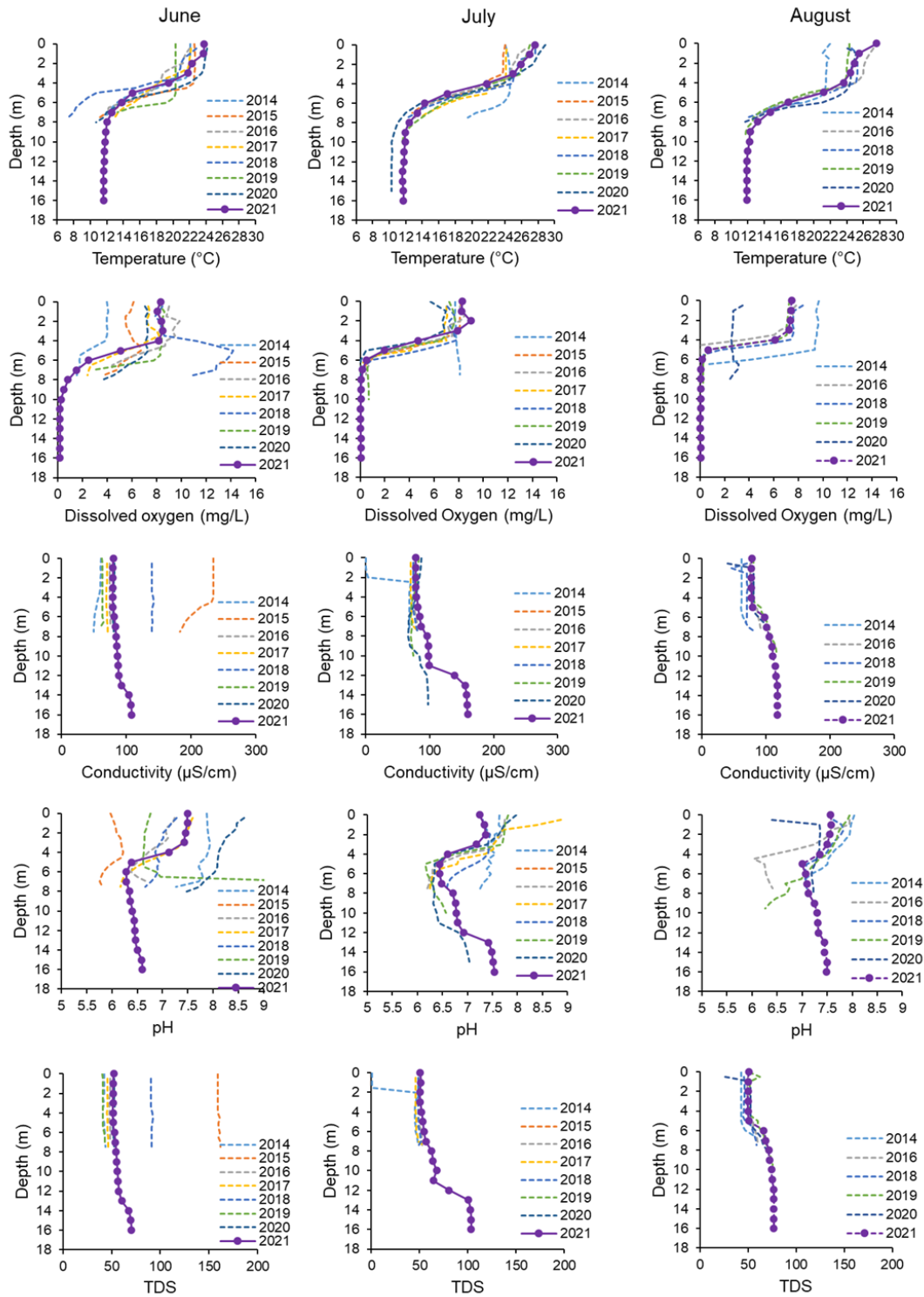
**Vertical Extinction Coefficient ( $k$ ):** The rate at which light attenuates with depth. Different wavelengths of light have different coefficients. Dependent on dissolved and particulate matter in lake water that may reflect or absorb light.

## [Appendix IV. Greenwater Laboratory Reports](#)

Included as separate files:

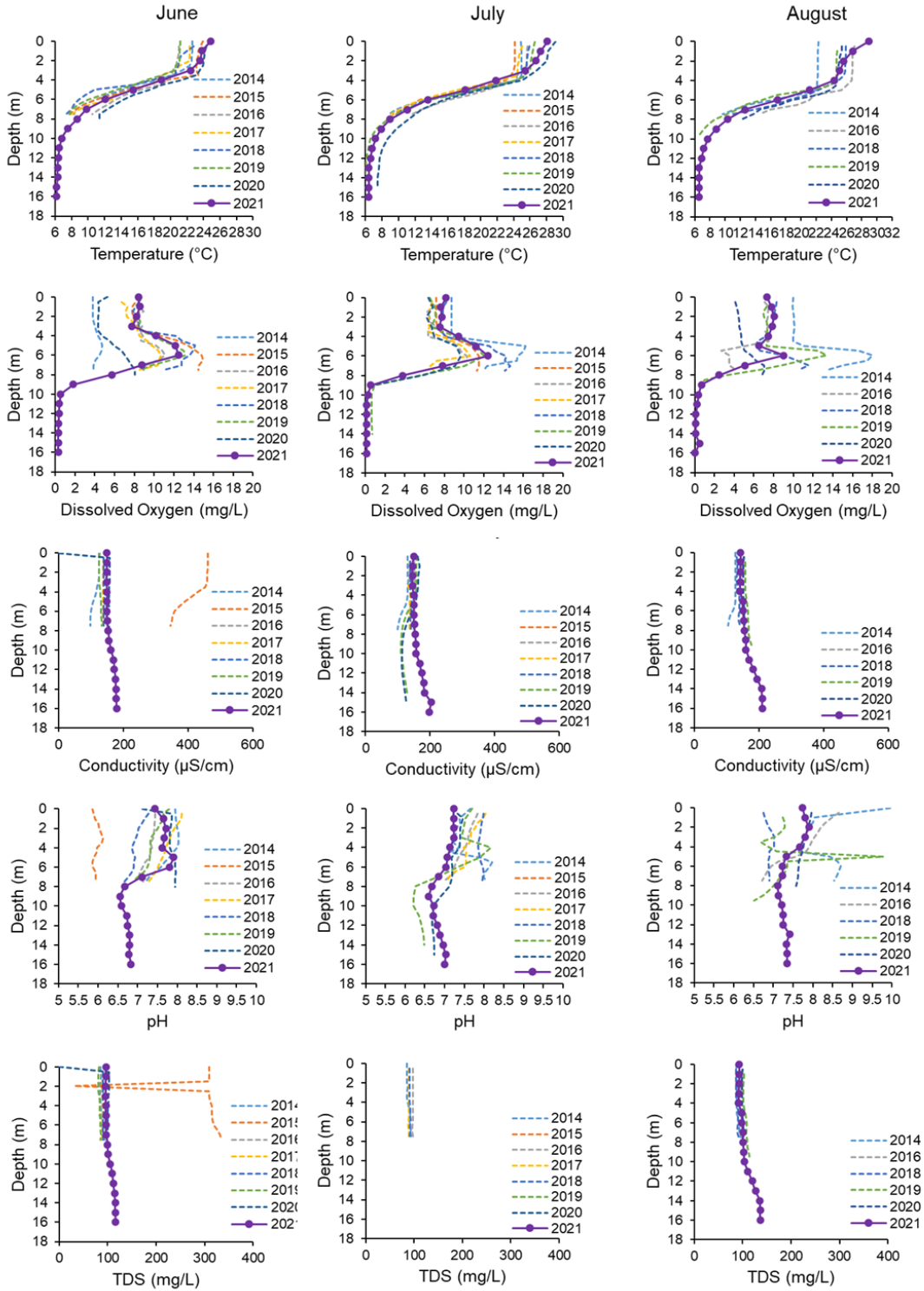
Lacawac Sanctuary PTOX Cyanobacteria Screen 210602

## Appendix V. Summer monthly profile data

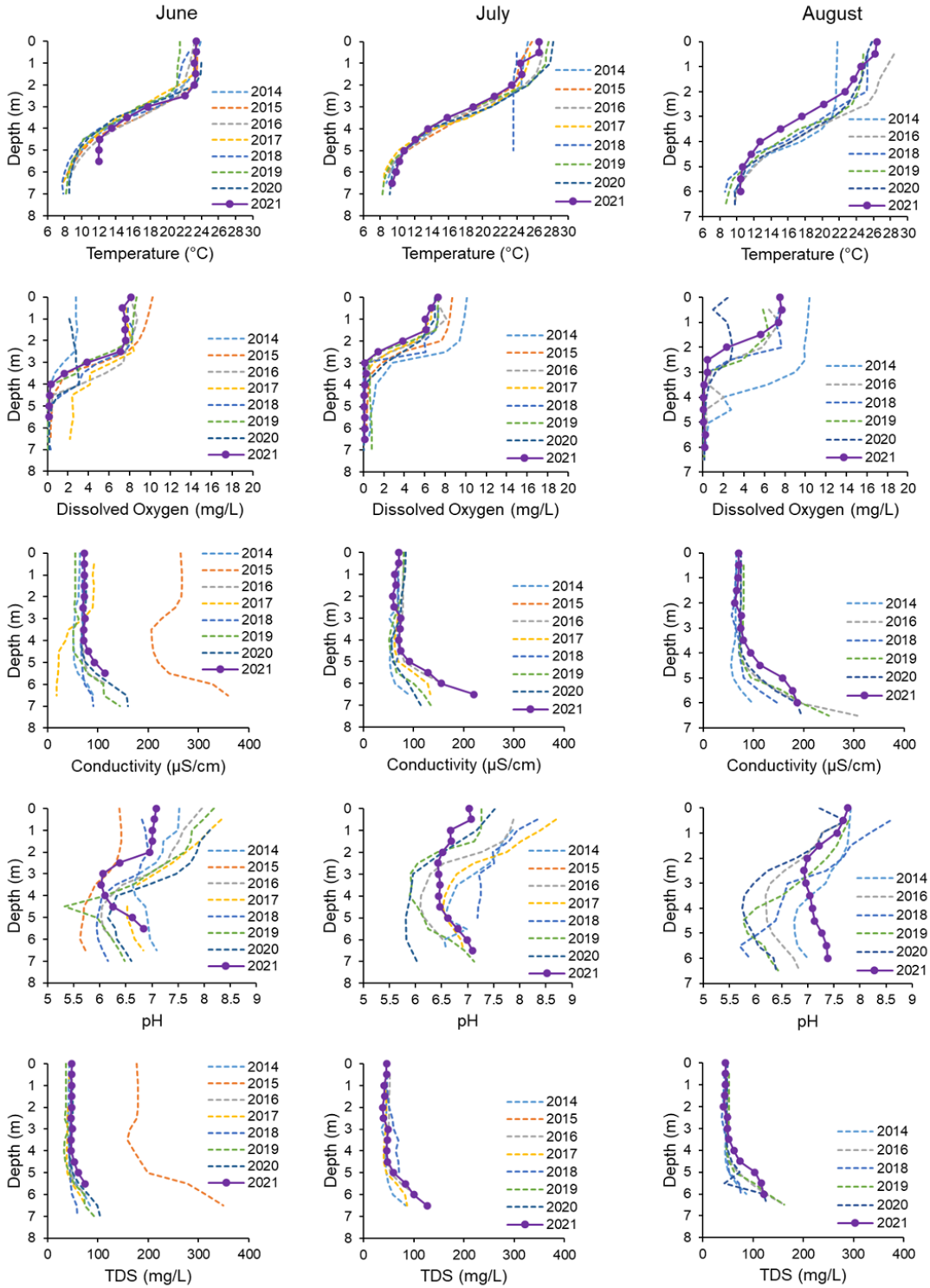


**Figure 24: June, July, and August profiles in Big Twin Lake since 2014. Profiles before 2021 (2019 for July) do not extend to full depth.**





**Figure 25: June, July, and August profiles in Little Twin Lake since 2014. Profiles before 2021 (2019 for July) do not extend to full depth.**



**Figure 26: June, July, and August profiles in Walker Lake since 2014.**