



The recent disappearance of a persistent *Planktothrix* bloom: Characterization of a regime shift in the phytoplankton of Sandusky Bay (USA)

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ABSTRACT

Sandusky Bay is the drowned mouth of the Sandusky River in the southwestern portion of Lake Erie. The bay is a popular recreation location and a regional source for drinking water. Like the western basin of Lake Erie, Sandusky Bay is known for being host to summer cyanobacterial harmful algal blooms (cHABs) year after year, fueled by runoff from the predominantly agricultural watershed and internal loading of legacy nutrients (primarily phosphorus). Since at least 2003, Sandusky Bay has harbored a microcystin-producing bloom of *Planktothrix agardhii*, a species of filamentous cyanobacteria that thrives in low light conditions. Long-term sampling (2003–2018) of Sandusky Bay revealed regular *Planktothrix*-dominated blooms during the summer months, but in recent years (2019–2022), 16S rRNA gene community profiling revealed that *Planktothrix* has largely disappeared. From 2017–2022, microcystin decreased well below the World Health Organization (WHO) guidelines. Spring TN:TP ratios increased in years following dam removal, yet there were no statistically significant shifts in other physicochemical variables, such as water temperature and water clarity. With the exception of the high bloom of *Planktothrix* in 2018, there was no statistical difference in chlorophyll during all other years. Concurrent with the disappearance of *Planktothrix*, *Cyanobium* spp. have become the dominant cyanobacterial group. The appearance of other potential toxigenic genera (i.e., *Aphanizomenon*, *Dolichospermum*, *Cylindrospermopsis*) may motivate monitoring of new toxins of concern in Sandusky Bay. Here, we document the regime shift in the cyanobacterial community and propose evidence supporting the hypothesis that the decline in the *Planktothrix* bloom was linked to the removal of an upstream dam on the Sandusky River.

1. Introduction

In recent decades, the western waters of Lake Erie (Laurentian Great Lakes) have been plagued by recurrent seasonal cyanobacterial harmful algal blooms (cHABs) that have compromised water quality and, in one instance, led to the shutdown of the municipal water supply to Toledo, Ohio, denying 400,000 people access to potable water (Bullerjahn et al., 2016; Steffen et al., 2017). In the western basin of Lake Erie, cHABs are typically comprised of microcystin producing *Microcystis* spp., driven by nitrogen and phosphorus (N and P) loadings from agricultural sources in the Maumee River watershed (Steffen et al., 2014a; Bullerjahn et al., 2016). Whereas *Microcystis* is the major genus affecting water quality in

the open waters of the western basin, *Planktothrix agardhii* blooms occur in rivers and in nearshore embayments (Rinta-Kanto and Wilhelm, 2006; McKay et al., 2018). These blooms also produce microcystins, often above the Environmental Protection Agency (EPA) contact advisory for recreational waters (Davis et al., 2015; McKay et al., 2018).

One such site that has been affected by *P. agardhii* (henceforth '*Planktothrix*') is Sandusky Bay, a shallow basin formed from a drowned river mouth (mean depth = 2.6 m; area = 162 km²) draining into southwestern Lake Erie (Fig. 1; Conroy et al., 2007). Sandusky Bay receives high N and P loadings from the Sandusky River, which flows through primarily agricultural areas (Conroy et al., 2007; Richards et al., 2009). The residence time in Sandusky Bay can vary from 8 to 81 days

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(Salk et al., 2018), similar to the residence time in Maumee Bay and the western basin, both of which are affected by *Microcystis* spp. (51 days; Millie et al., 2009). The differences in cHAB composition in Sandusky Bay (*Planktothrix*) versus Lake Erie (*Microcystis*) have been attributed in part to different nutrient availability in the two sites. Specifically, in Sandusky Bay the availability of combined N decreases as the summer bloom progresses, starting with high concentrations (50–600 μM) of dissolved inorganic nitrogen (DIN; including nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) in June and July, followed by low ($< 5 \mu\text{M}$) to undetectable DIN concentrations in August–October. This is mainly due to a decrease in nitrate mediated by high rates of sedimentary denitrification (Davis et al., 2015; Salk et al., 2018). Whereas N losses are in part due to assimilation by bloom biomass, N is not drawn down stoichiometrically with P, indicating that denitrification is the more dominant process, and denitrification rates are ten-fold higher than documented in western Lake Erie (Small et al., 2016; Salk et al., 2018). Given that *Planktothrix* is a better scavenger for N than is *Microcystis* (Hampel et al., 2019), we have proposed that N availability is an important driver in differentiating between the establishment of *Microcystis* and *Planktothrix* cHABs (Hampel et al., 2019). In fact, *Planktothrix* is common in lakes with low bioavailable N and low N:P (Rückert et al., 1997), consistent with the conditions prevailing in Sandusky Bay in mid summer to autumn, where N:P can fall below Redfield (Salk et al., 2018). While the bloom is established during springtime when there is a higher N:P, *Planktothrix* blooms can form at temperatures lower than those ideal for other cyanobacteria (Oberhaus et al., 2007) and then persist in N-limited waters through the summer months (Hampel et al., 2019). Overall, low N concentrations by the end of summer and elevated, albeit variable, concentrations of soluble reactive phosphorus (SRP) provided through internal loading (Davis et al., 2015; Salk et al., 2018) promote seasonal N limitation in Sandusky Bay. Prolonged presence of *Planktothrix* bloom biomass from spring through late fall was also attributed both to growth across a broad temperature range and acclimation to low light intensity (Oberhaus et al., 2007). Shallow (2–3 m), well-mixed Sandusky Bay leads to suspended sediment particles that create turbidity and low light conditions (Scheffer et al., 1997). Together, the environmental conditions of Sandusky Bay were consistent with other studies documenting the success of *Planktothrix* in forming persistent freshwater cHABs. Indeed, such conditions promoting *Planktothrix* blooms have been documented in lakes such as Grand Lake St. Marys, Ohio (USA), a nearby inland lake (Filbrun et al., 2013; Steffen et al., 2014b), and Independence Reservoir, Ohio (McKay et al., 2018).

Planktothrix blooms were regular, predictable yearly events since

they were first documented and characterized in 2003 (Rinta-Kanto and Wilhelm, 2006). However, in 2020, the *Planktothrix* cHAB did not occur, and has yet to recur to date (through 2023). In this paper, we examine the changes in the cyanobacterial communities and physicochemical variables and propose hypotheses to explain the sudden regime shift. Specifically, the removal of an upstream dam is temporally linked to the decline in the bloom. Detection of residual microcystins and *Planktothrix* DNA in exposed soils previously submerged by the dam reservoir suggests that the reservoir harbored a seed stock of *Planktothrix* that fed the bloom in the Bay downstream.

2. Methods

2.1. Study area and water processing

Sandusky Bay is a shallow basin (mean depth = 2.6 m; area = 162 km^2) formed from the mouth of the Sandusky River draining into southwestern Lake Erie (Fig. 1; Conroy et al., 2007). The bay is divided into two parts: the Inner Bay, which starts at Muddy Creek and extends to the Edison Bridge, and the Outer Bay, which extends from the Bridge to the Bay mouth at Cedar Point. Starting in 2015, four sites (i.e., ODNR4, ODNR6, ODNR2, ODNR1) were consistently sampled with the assistance of the Ohio Department of Natural Resources (ODNR) and additional sites have since been included (10 total; Fig. 1). In 2020, the frequency of sampling was increased from biweekly to weekly. Additional samples were taken in the floodplain of the Sandusky River to test for the presence of residual toxins and cyanobacteria in a former reservoir upstream from the Bay.

2.2. Physicochemical data and water sampling

During each sampling trip, physicochemical variables were measured at approximately the same time of day for each site by using a calibrated Model 600 QS water quality probe (2017–2019) and a EXO2 Multiparameter Sonde (YSI Inc., Yellow Springs, OH, USA) (2020–2022) to measure surface water temperature, dissolved oxygen concentration, pH, turbidity, and conductivity. Light transmission in the water column was measured with a LI-COR submersible quantum sensor (Lincoln, NE, USA). In addition, microbial biomass was collected by filtering approximately 100 mL of lake water through a 0.22- μm pore size Sterivex filter cartridge (Sigma Aldrich, St. Louis, MO). After evacuating residual water, RNAlater solution (Invitrogen, Thermo Fisher Scientific Inc, Waltham, MA) was added to fill the cartridge, which was then immediately transferred to liquid nitrogen while in the field; filters were

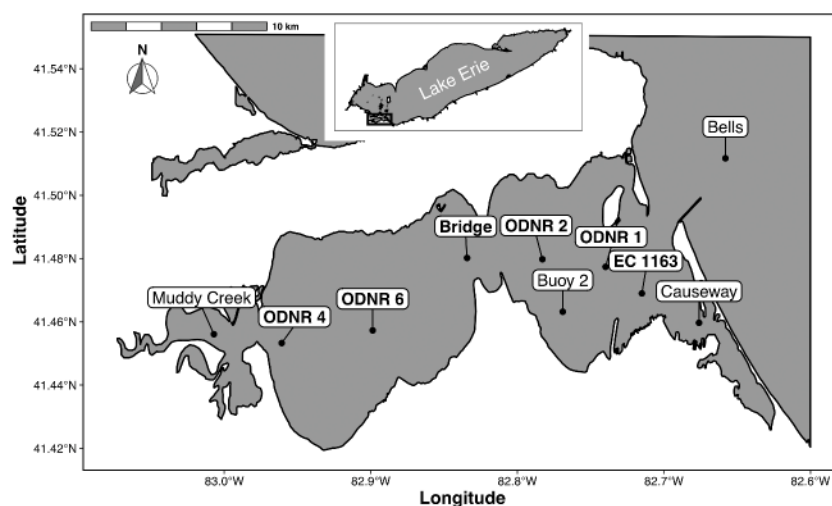


Fig. 1. Location of sampling sites located in Sandusky Bay, Lake Erie. The inset map shows location of the Bay with respect to the whole lake. Sampling sites in bold are those used for 16S rRNA sequencing.

stored in -80°C freezers once returned to the lab. Samples for nutrient analysis of dissolved (0.22- μm filtrate) and total (whole water) fractions were collected at each site and placed on ice in the field until storing at -20°C . Nutrient samples were analyzed by SEAL AA3 autoanalyzer at The Ohio State University's Franz Theodore Stone Laboratory using EPA-approved standard methods (APHA et al., 1998). Microcystin equivalents were measured in the particulate and dissolved forms using the Abraxis Microcystins-ADDA 194 ELISA immunoassay (Abraxis LLC; Warminster, PA; EPA Method 546). Real-time conductivity measurements were also obtained from an EXO-2 sonde permanently deployed at the Bridge site, and data were downloaded from the Great Lakes Observing System (GLOS; see <https://seagull.glos.org>).

2.3. DNA extraction and library preparation

Microbial DNA from Sterivex filters was extracted using the DNeasy PowerWater Kit (Qiagen, Germantown, MD, USA). Sample filter cartridges were opened with a sterilized pipe cutter and the filter was removed prior to extraction per the manufacturer's instructions. Extracted DNA samples were quantified using a NanoDrop One micro-volume UV-Vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA USA), and DNA samples that had a concentration of at least $20\text{ ng }\mu\text{L}^{-1}$ were selected for further analysis and stored at -80°C . Samples from 2018 to 2020 were sequenced by HudsonAlpha Discovery Life Sciences (Hudson, AL, USA) using Illumina MiSeq V3 chemistry (Illumina, San Diego, CA) with $2 \times 300\text{ bp}$ reads. Samples from 2021 to 2022 were sequenced by University of Minnesota Genomics Center using Illumina MiSeq V3 chemistry with $2 \times 300\text{ bp}$ reads. Microbial communities from both sequencing efforts were characterized using a dual-index sequencing approach (Kozich et al., 2013) in which the V3-V4 region of 16S rRNA gene was amplified by PCR using 341F and 785R primers described in Klinkworth et al., 2013.

2.4. Sequence processing and microbial community analysis

Sequences were demultiplexed using CASAVA (Illumina). All downstream sequence processing was performed using the DADA2 version 1.26.0 workflow (Callahan et al., 2016) in R (R Core Team, 2023, version 4.3.1). We chose to use ribosomal gene amplicon sequence variants (ASVs) to correct for the potential sequencing artefacts and limitations associated with operational taxonomic units (OTUs; Callahan et al., 2016). Reads were trimmed on the 5' to remove primer sequences and the 3' to remove low-quality nucleotides. Read pairs were then denoised using a DADA2-generated error model, merged, and *de novo* chimeras were removed. The final output consisted of the final ASV sequences of 16S rRNA gene reads and an ASV table of the number of reads per ASV for each sample. The number of reads per ASV was used to calculate relative abundance. ASVs were then assigned using RDPtools and RDP release 11.5 classifiers (Wang et al., 2007) and taxonomy selected against the SILVA database (version 138.1). The resulting ASV table was used to generate relative abundance taxa bar plots using phyloseq and ggplot R packages. Principal coordinate analysis was performed on a Bray-Curtis dissimilarity matrix. Sample libraries that failed to yield an adequate number of high-quality reads were excluded from further analyses.

2.5. Ballville Dam, Sandusky River soil extraction and processing

The Ballville Dam was constructed on the Sandusky River in 1913 for hydroelectric power generation and decommissioned in 1959. After decommissioning, the reservoir was used as a drinking water source of the City of Fremont, OH until 2013, when the city switched to an alternate reservoir as their water source (see: greatlakesnow.org/2022/12/ballville-dam-removal-history-trends-impacts). In October 2018, the Ballville Dam on the Sandusky River (upstream from the Bay) was demolished to improve fish habitat and spawning. In April 2023,

samples were taken from sediment deposited upstream of the dam site in the former Ballville Reservoir. Soil samples were collected by pushing a clear round plastic tube into the soil until compaction, which were then frozen at -20°C until further processing. Samples taken at each of the four sites were extracted for DNA and microcystins. After thawing, each core was measured and then segmented into 7 cm sections that were then homogenized by hand. After homogenization, the soil was weighed into 40 g portions for microcystin analysis and triplicate 1.2 g portions for DNA for each section. Soil samples for DNA were stored at -80°C until further processing. The 40 g of soil was added to a flask with 100 mL of deionized water and set to a slow boil for 5 min. After cooling, the water was passed through a 0.22- μm Sterivex filter and the filtrate stored at -20°C . The concentrations of microcystins were measured, as described above in Section 2.2.

The 1.2 g of soil was thawed and weighed again for DNA extraction using a DNeasy PowerSoil Pro Kit (Qiagen, Germantown, MD, USA) per the manufacturer's instructions. Real-time PCR was then performed to quantify *Planktothrix* abundance, as outlined in McKindles et al., 2021. In brief, equal volumes of each extracted DNA sample and forward and reverse primers (400 nM) were run with PowerUp SYBR Green Master Mix (Thermo Fisher Scientific, Waltham, MA USA). After an initial activation step at 50°C for 2 min and a denaturing step at 95°C for 2 min, 40 cycles were performed as follows: 15 s at 95°C , 30 s at 55°C and 60 s at 72°C . The efficiency of the rpoC1 primer set is 97.1% described in McKindles et al., 2021.

2.6. Statistics

Data analysis was done using RStudio version 2023.09.0-463 working on R version 4.3.1 (2023-06-16). Furthermore packages: Tidyverse (version 2.0.20), DADA2 (version 1.28.0), Vegan (version 2.6-4), phyloseq (version 1.44.0), rstatix (version 0.7.2), and stats (version 4.3.1) were used for all data processing, statistical analysis, and graphing. Statistical significance was assessed using t-test with Bonferroni corrections on physicochemical parameters and total phosphorus. Assumptions for the analysis were tested using qq-plots, outlier checking, and Shapiro-Wilk test for instance with >0.05 being normally distributed data. Non-normally distributed parameters TN:TP, TN, chlorophyll-a and microcystins were analyzed using Kruskal-Wallis, Dunn test, and Wilcoxon test corrected with Bonferroni on log transformed data. All statistics were run with data from samples collected between days of year 160–230. Linear regressions were used on the TP and TN data using 95% confidence intervals for trend analysis.

Community relative abundances were compared using ADONIS permutational multivariate analysis of variance, Kruskal-Wallis one-way analysis of variance, Wilcoxon rank sum, and was corrected using the Benjamini-Hochberg protocol. All statistical significance was determined at < 0.05 threshold and the same sites were included for all analysis (ODNR4, ODN6, Bridge, ODN2, Buoy 2, ODN1, and EC1163).

3. Results

3.1. Physicochemical data

Physicochemical parameters were collected throughout the sampling period to track any potential changes over time. Water temperature between day of year 160 to 260 range from 17.25°C to 29.22°C (Fig. 2). In late June of both 2019 and 2020 there were $3\text{--}4^{\circ}\text{C}$ decreases in temperature before increasing above 25°C (Fig. 2). Temperatures then remained warmer than previous years before decreasing in line with other years as the season changed from summer to fall. Overall, there was no statistical difference in temperature from year to year with respect to 2018. Throughout 2017–2022, pH ranged from 8.3 to 10.7, with a mean of 9.2, and pH had the highest average in 2017 (10.3, 9.2, 9.5, 9.1, 9.1, and 8.6; 2017–2022 respectively). Pairwise comparisons of

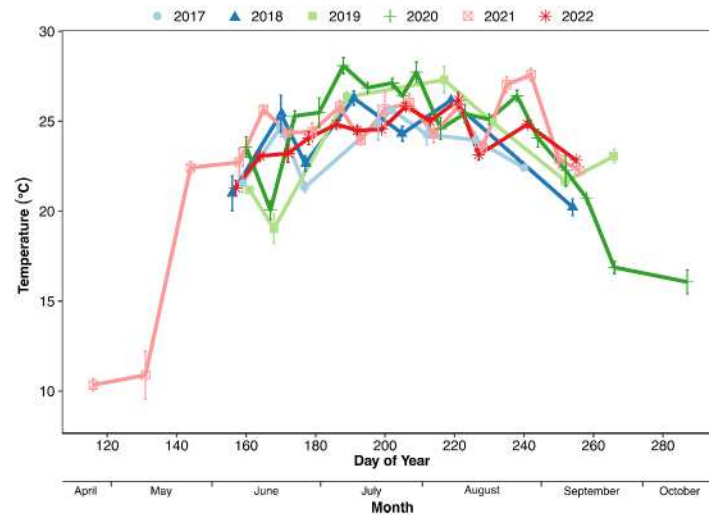


Fig. 2. Water temperature measurements throughout the years 2017–2022. Error bars represent standard deviation between sites. Each point represents 7 sites within the bay.

pH with respect to 2018 revealed that 2019 and 2022 were significant ($p = 3.03\text{E-}04$, $2.06\text{E-}12$; respectively) and 2020–2021 were not significant ($p = 1$, $3.44\text{E-}01$; respectively). Conductivity remained similar over 2018–2022, however pairwise comparisons indicate that 2018 was significantly different from years 2021 and 2022 ($5.60\text{E-}05$, $3.06\text{E-}06$; respectively), 2019 being different from years 2020–2022 ($p = 3.00\text{E-}02$, $4.52\text{E-}05$, $1.75\text{E-}05$; respectively; Supplemental Fig. 1; Supplemental Table 1). Additionally, 2021 had the largest spike in conductivity at the end of June before declining to similar values of other years (Supplemental Fig. 1). In comparison to 2018, years 2021 and 2022 had significantly higher conductivity ($p = 2.24\text{E-}05$, $1.22\text{E-}06$; for 2021–2022 respectively). Measurements of water transparency taken as Secchi disk depth readings revealed that 2019 had overall lower light transmission than all other observed years. Years 2020 and 2021 had large spikes in water transparency in the beginning of June before stabilizing within the same range as other years, besides 2019 (Supplemental Fig. 2).

3.2. Nutrients

Dissolved inorganic nitrogen (DIN) decreases throughout the year, which is reflected in a decrease in TN:TP most years (Fig. 3, also see Salk et al., 2018). In years 2018–2022, TN:TP ratios fall within the range previously documented for high bloom years 2015 and 2016 (Salk et al., 2018). Total phosphorus (TP) within the Bay trended downward through the years (Supplemental Figs. 3 and 4). Comparing the years against year 2018, 2019–2022 all had significantly lower TP concentrations throughout the whole year ($p = 4.72\text{E-}04$, $1.48\text{E-}16$, $9.32\text{E-}11$, $1.33\text{E-}08$; respectively). Furthermore, total phosphorus concentrations from 2018 were significantly different from the years 2019–2022 ($p = 1.48\text{E-}12$). Nonetheless, such changes in measured TP are not reflected in changes in springtime P loads in the Sandusky River 2018–2022 (N. Manning, National Center for Water Quality Research, personal communication; see NCWQR 2022). By comparison, total nitrogen (TN) in the Inner Bay trended upward, while the Outer Bay trended

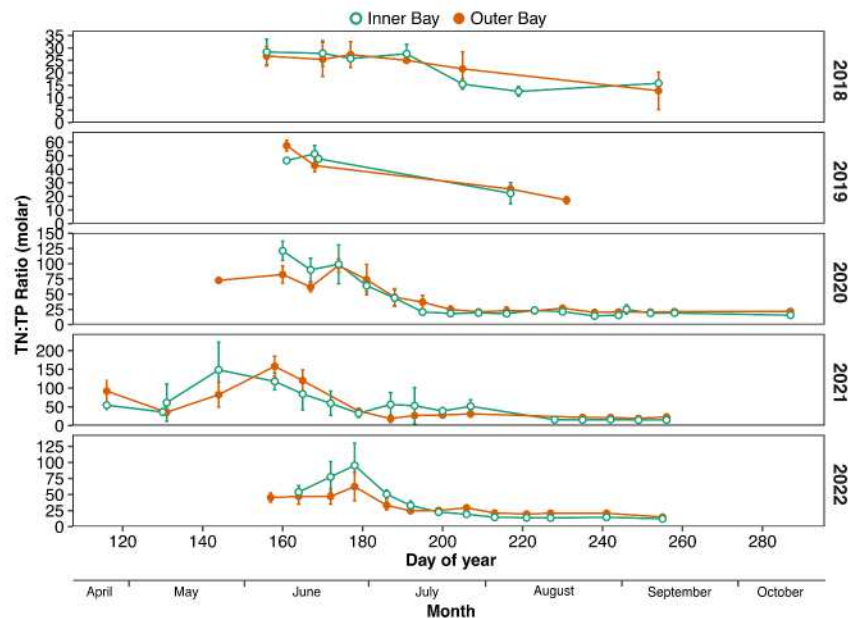


Fig. 3. Total nitrogen to total phosphorus ratios throughout the year. Each panel represents one year of sampling; lines represent either Inner Bay or Outer Bay sites. Note the differences in scale between years, reflecting interannual variation in springtime nutrient loads. Each point represents 3 sites for inner bay and 4 sites for outer bay.

downward. Averaging over the entire sampling seasons, total nitrogen was significantly different from 2018 to 2020 and 2022 ($p = 1.17\text{E-}05$, $3.00\text{E-}03$; respectively) but not different from 2019 to 2021 ($p = 5.70\text{E-}02$, $1.36\text{E-}01$; respectively). As a result of decreasing TP trends with slight increase in TN, there was an increase in the TN:TP ratio through years 2018–2022 (Fig. 3). TN:TP ratios in 2019–2021 were all significantly different ($p = 3.44\text{E-}05$, $6.00\text{E-}03$, $5.28\text{E-}05$; respectively) compared to 2018.

3.3. Chlorophyll-*a*

Chlorophyll-*a* concentrations were used as an indicator for phytoplankton biomass. Extracted chlorophyll-*a* concentrations ranged from $1.71 \mu\text{g L}^{-1}$ to $323.22 \mu\text{g L}^{-1}$ and a mean of $69.2 \mu\text{g L}^{-1}$ throughout the six years, and the Inner Bay were routinely higher than the Outer Bay apart from 2019 (Fig. 4). With respect to the Inner Bay and 2018, years 2019 through 2022 were significantly different than in 2018 ($p = 3.66\text{E-}05$, $2.20\text{E-}07$, $1.07\text{E-}04$, and $1.31\text{E-}05$; respectively). By contrast, the Outer Bay was only significantly lower in year 2020, 2021, and 2022 ($p = 4.92\text{E-}05$, $1.06\text{E-}04$, and $6.92\text{E-}05$; respectively). Year 2018 had the highest chlorophyll and was significantly higher than years 2019–2022 regardless of the location in the Bay (Supplemental Table 2).

3.4. Microcystin toxins

The provisional guideline values (GVs) for microcystins exposure (lifetime drinking-water GV: $1 \mu\text{g L}^{-1}$; short term drinking-water GV: $12 \mu\text{g L}^{-1}$; recreational water GV: $24 \mu\text{g L}^{-1}$) were set by the World Health Organization (WHO; World Health Organization, 2020). Years 2017 and 2018 had higher concentrations of total microcystins (sum of particulate and dissolved fractions) throughout the year that routinely exceeded the short-term drinking water standard (Fig. 5). In 2019 there was a spike in total microcystins, followed by a decline through the rest of the year (Fig. 5). Years 2019–2022 saw significantly lower amounts of total microcystins compared to 2018 and most dates fell below lifetime drinking water guideline ($p = 6.49\text{E-}16$; Fig. 5). Pairwise comparisons excluding 2017 yielded similar results with all pairs being significantly different except for 2018–2019, 2019–2022, and 2020–2021 ($p = 3.09\text{E-}01$, $7.43\text{E-}02$, 1 , respectively; Supplemental Table 3).

3.5. Microbial community composition

16S rRNA amplicon sequencing of extracted DNA samples revealed a distinct shift in the cyanobacteria during the study period (Fig. 6). In 2018, *Planktothrix* composed an average of 97% of the cyanobacterial taxa, consistent with previous analyses (Rinta-Kanto and Wilhelm, 2006; Salk et al., 2018; Hampel et al., 2019). There were 71 assigned sequence variants corresponding to *Planktothrix*. In 2019, *Planktothrix* remained the dominant taxon, but other genera (e.g., *Aphanizomenon*, *Dolichospermum*, and *Cyanobium*) were present in substantial abundances (Fig. 6B). In 2020–2022, *Cyanobium* replaced *Planktothrix* as the dominant genus (Fig. 6B). Years 2020–2022 began with a brief emergence of an *Aphanizomenon* bloom in the beginning of the summer; 2021 had a smaller transient bloom relative to 2020 and 2022 (Fig. 6B). 2019 was the first year in which *Aphanizomenon* was first shown in the Bay in high relative abundance. From 2020 and 2022, *Planktothrix* never exceeded 5% relative abundance.

As might be expected, species diversity, as measured by Shannon's Index, increased in years without *Planktothrix* blooms. Results from a Kruskal-Wallis rank sum test indicate significantly different between all years ($p = 2.2\text{E-}16$). All years are significantly different from one another except for 2021–2022 ($p = 5.69\text{E-}01$, pairwise Wilcoxon rank sum comparisons; Supplemental Table 4). Changes in the community structure are further visualized by a principal coordinate analysis (PCoA) with Bray-Curtis dissimilarities (Fig. 7). This ordination plot indicates that the progressive change in Sandusky Bay's cyanobacterial community from 2018 (*Planktothrix*-dominated) to 2022 (primarily *Cyanobium*), with years 2018 and 2020–2022 forming distinct clusters and 2019 appearing as a transitional community state. This is further supported by ADONIS (permutation-based analysis of variance) where pairwise comparisons of communities from each individual year were significantly different from one another with an overall difference of $p = 1.00\text{E-}5$.

3.6. Evidence of potential upriver source of planktothrix

Given that the dramatic shift in the cyanobacterial communities occurred shortly after removal of the Ballville Dam, we asked whether the original reservoir could have served as an upstream source of *Planktothrix* for the Bay. Consequently, we analyzed soil cores taken from floodplain sites corresponding to the original reservoir bottom, and

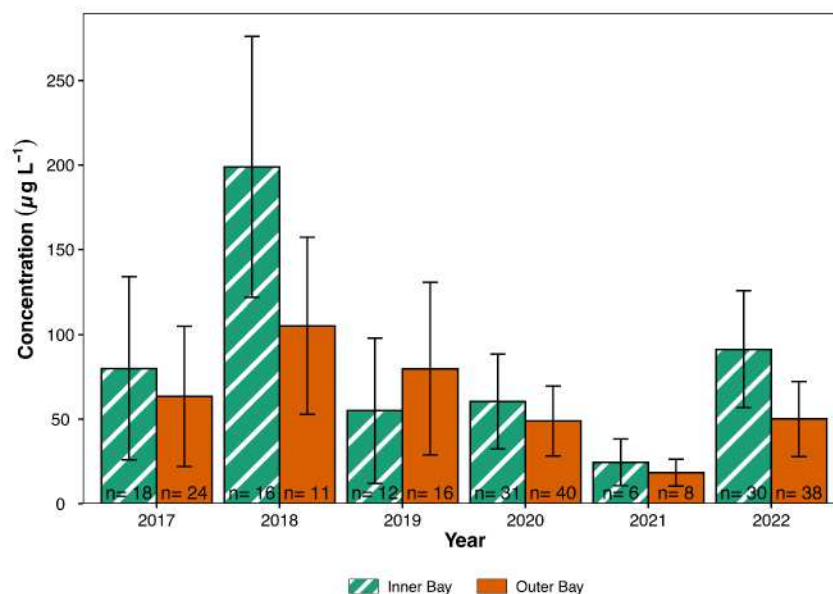


Fig. 4. Extracted chlorophyll-*a* concentrations, averaged over all sites for all dates each year. Error bars are standard deviation. The number of measurements for each year is indicated at the bottom of the bars.

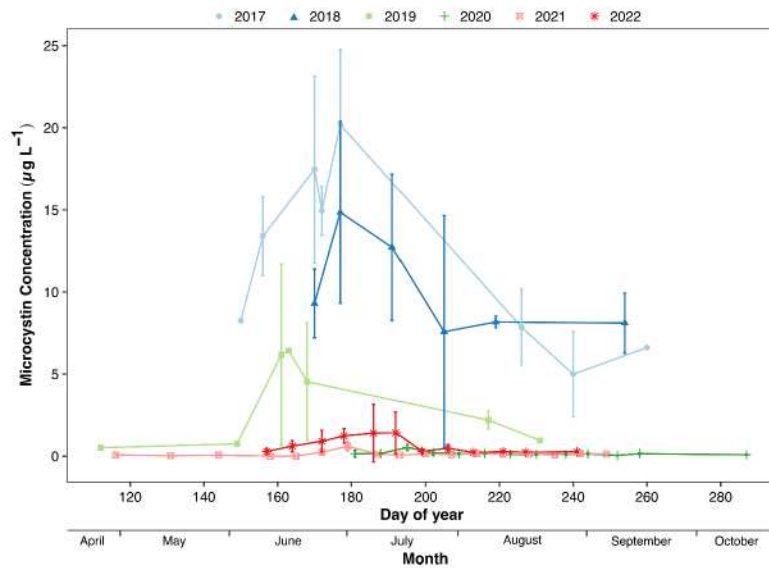


Fig. 5. Total microcystin concentrations from 2017 to 2022, measured by ELISA following EPA methods. Each point represents all sites with greater than 0 concentration on their respective DOY.

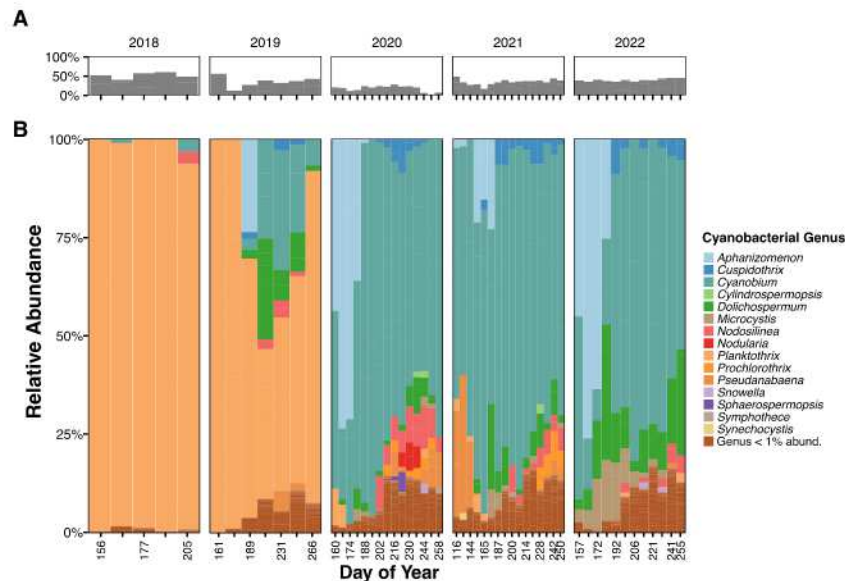


Fig. 6. Cyanobacteria annotated at the genus level. Panel A is the percent of the total 16S reads that were cyanobacteria. Panel B is the cyanobacterial community at the genus level with taxa present at less than one percent of the total abundance was grouped together. The x-axis is the day of year (DOY), and the y-axis shows relative abundance. Each site was merged and averaged into the same DOY for analysis and each panel is a different year.

these cores were analyzed for residual microcystins by ELISA and *Planktothrix* DNA through qPCR (Fig. 8). All sites had detectable microcystin concentrations, with Site 4 having the highest average dissolved toxin ($2.45 \pm 1.91 \text{ ng g}^{-1}$ sediment). Site 3 had the next highest average with $1.98 \pm 0.22 \text{ ng g}^{-1}$ followed by Site 1 and Site 2 with 0.75 ± 0.21 and $0.64 \pm 0.19 \text{ ng g}^{-1}$ respectively. With respect to *Planktothrix* DNA, the soil cores from Sites 1 and 4 contained detectable residual *Planktothrix* 16S rRNA gene copies above the limit of detection (LOD), with 3.63 and 152.99 copies g^{-1} sediment for Sites 1 and 4, respectively.

4. Discussion

This study documents a major shift in cyanobacterial community structure in a CHAB-affected freshwater estuary. The consistent, microcystin-producing *Planktothrix* bloom that once dominated

Sandusky Bay has been replaced by a community largely consisting of *Cyanobium*. This work outlines the importance of long-term system-wide monitoring of both biological and physicochemical parameters so that the stability of a system can be assessed, and the data used to inform future management decisions.

Despite the notable changes in cyanobacterial community composition, the system is still eutrophic, with chlorophyll concentrations typically averaging above $50 \mu\text{g L}^{-1}$ throughout the summertime bloom period. Since the dominant cyanobacteria has shifted from potentially toxigenic *Planktothrix* to largely nontoxic picocyanobacteria, microcystin concentrations have declined to well below the WHO contact advisory standard. Nonetheless, the transient appearance of other potentially toxic genera (e.g., *Aphanizomenon* spp.) warrants future monitoring of other cyanotoxins, such as saxitoxins and anatoxin.

Explanations for the change in phytoplankton community composition could be attributed to possible changes in nutrient concentrations

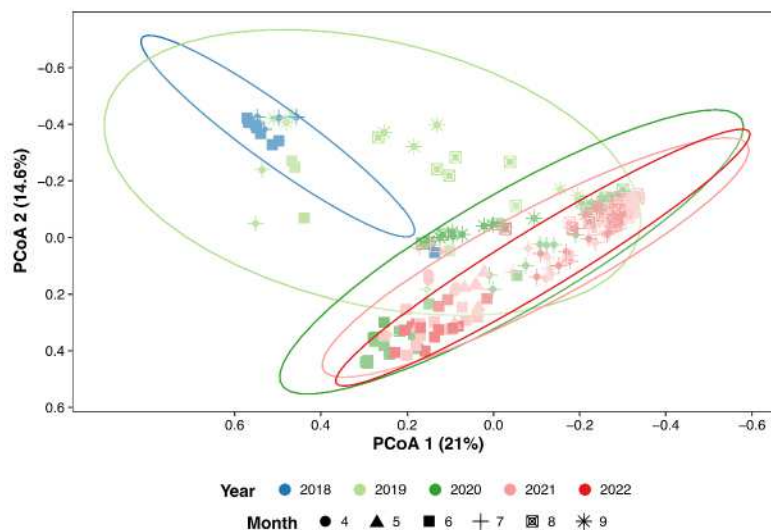


Fig. 7. Principal coordinate analysis (PCoA) plot showing the compositional differences (Bray-Curtis dissimilarities) of cyanobacterial communities between sample years and months. Years are indicated by color, shapes indicate month of the year, and the ellipses are drawn showing 95% confidence intervals assuming multivariate normal distribution.



Fig. 8. Aerial map images of Sandusky River at the Ballville Dam, Ballville, Ohio, retrieved from Google Maps. (A) The historical map from 2015 before the dam was removed. (B) Ballville Dam site in 2021, two years after removal of the dam. Sites were only sampled post dam removal and were chosen based on the historical location of pools.

and loading. It was investigated whether record high water levels in Lake Erie during 2020–2021 (US Army Corps of Engineers, 2022) was moving possibly lower-nutrient, low-conductivity water into the Bay from the open lake. However, conductivity values trended higher during this period (Supplemental Fig. 1), indicating that water chemistry in the Bay was primarily influenced by riverine and autochthonous factors and incursion of lake water had relatively minor impact. Patterns in declining TN:TP ratios as summer progressed were observed each year, similar to previous studies (Salk et al., 2018), reflecting high rates of denitrification in the Bay. Prior studies tracking TN:TP demonstrated that these are the prevailing nutrient conditions observed during *Planktothrix* blooms occurring prior to 2018 (Davis et al., 2015; Salk et al., 2018; Hampel et al., 2019). During the period from 2018 to 2022, TP in Sandusky Bay did decrease significantly, increasing early summer TN:TP ratios. This was not reflected trends in springtime TP loads in the Sandusky River, measured upstream of the previous dam (NCWQR, 2022), suggesting internal loading in the Bay or in the lower stretch of the river. The elevated TN:TP ratio in Sandusky Bay from 2019 to 2023 is still lower than that of the Bells site outside the Bay that is representative of the western basin at large (see <https://ryansw22.github.io/github.io/> for the complete long term data sets of all sites).

Coincident with the decline in *Planktothrix* was the removal of the

Ballville Dam in the Sandusky River that empties into Sandusky Bay. We investigated whether dam removal and the loss of the Ballville Reservoir may have played a role in the change in cyanobacterial community, possibly by eliminating an upstream source of *Planktothrix* in the reservoir that could have seeded the bloom downstream in the Bay. Detection of both toxin and *Planktothrix* 16S rDNA in the sediment from the former reservoir site provides some evidence of this potential mechanism. Of course, if the dam did play a role in bloom formation, the effects of its removal may have also had indirect effects through changes in nutrient (P and N) availability that may have favored the switch to *Microcystis*. The documented changes in TP and TN also occurred coincident with dam removal.

Decreases in residence time following dam removal could also play a role in decreasing the bloom in the Bay, switching from a lentic system favoring cyanobacterial growth to more lotic conditions. A longer-term hydrologic study is warranted that considers both seiche effects and variability in Sandusky River flow during wet and dry periods to determine if the residence time is significantly shorter than the historical record. Furthermore, it remains to be seen whether the increase in TN:TP ratio documented post dam removal are transient or more permanent and whether that can be linked to the changes in the phytoplankton community. Future monitoring efforts are planned to continue in

Sandusky Bay at least through 2026, and issues regarding residence time and nutrient availability will be addressed through such a long-term study.

While the removal of the Ballville Dam was an attempt to improve fish habitat (US Department of Interior, 2014), the removal may have had unintended positive consequences for waters below the dam. In other locations, dam removal has resulted to varying degrees in reverting negative water quality impacts and improving dissolved oxygen concentrations (Tuckerman and Zawiski, 2007; Abbott et al., 2022). Biotic communities including macroinvertebrates may benefit from the removal by reverting the aquatic habitat from a lentic system back to a free-flowing system. Some have reported dramatic changes in taxonomic composition in benthic communities downstream of other dam removal projects (Bushaw-Newton et al., 2002; Stanley et al. 2002). Changes have also been documented for sediment microbial communities (e.g., Kan et al., 2023), but somewhat surprisingly there are scarce studies of phytoplankton upstream and downstream of damming and dam removal. Collectively, dam removal typically results in short-term community shifts that stabilize over time (Tullos et al., 2014; Bellmore et al., 2019), but it is unclear whether Sandusky Bay has now reached a stable state or continues in transition. Nonetheless, our characterization of the phytoplankton and microbial communities in Sandusky Bay has revealed potential positive effects throughout the food web that can possibly be documented in other systems. Looking toward the future, it may benefit managers to look at the input waters for dams as a potential incubator of toxic cyanobacteria and assess whether dam removal might help mitigate blooms downstream.

Data availability: 16S rRNA gene sequences were deposited in the NCBI Sequence Read Archive under project number PRJNA1051097. Environmental metadata for years 2018 and 2019 are available as part of the Sandusky Bay database through BCO-DMO dataset 755,348.1, 814,593.1 (Bullerjahn and McKay, 2020). Additionally, data is available on GitHub (<https://github.com/ryansw22/SanduskyBayRegimeShift.git>) and can be provided upon request.

CRediT authorship contribution statement

Ryan S. Wagner: Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Michelle J. Neudeck:** Writing – review & editing, Investigation, Data curation. **Alexis E. Heath:** Writing – review & editing, Investigation, Data curation. **Katelyn B. Barker:** Writing – review & editing, Investigation, Data curation. **Katelyn M. Brown:** Writing – review & editing, Investigation, Data curation. **Seth Buchholz:** Writing – review & editing, Investigation, Data curation. **Christopher S. Ward:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **George S. Bullerjahn:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.hal.2024.102656](https://doi.org/10.1016/j.hal.2024.102656).

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