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PRIMARY RESEARCH PAPER

Effects of freshwater input on nutrient loading, phytoplankton biomass, and cyanotoxin production in an oligohaline estuarine lake

Sibel Bargu · John R. White · Chunyan Li · Jessica Czubakowski · Robinson W. Fulweiler

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Abstract Pulsed river water events can increase nutrient levels potentially translating into enhanced primary production, phytoplankton community shifts, and bloom formation. The Bonnet Carré Spillway is a managed river diversion which can be used to redirect a significant amount of Mississippi River water into Lake Pontchartrain, reducing the risks of flood in the downstream communities during runoff seasons. We investigated nutrient enrichment and consequent changes in phytoplankton biomass, including toxic species in Lake Pontchartrain during and after a 1-month Bonne Carré Spillway opening in 2008. Water samples were collected along a 30 km transect. A freshwater plume was found to have formed by the strong river input that had limited mixing with the lake during the opening. The plume and lake water gradually mixed together after the Spillway was closed, indicated by the reduction of the horizontal salinity gradient. The river pulse increased the lake nitrate and dissolved reactive phosphorus concentrations to more than five times the lake background in the plume stations. Nutrient concentrations decreased rapidly after the Spillway closure as the plume dissipated. Diatoms and chlorophytes dominated the system during the opening. After the Spillway closure, there was a shift over time from diatom dominance to toxic cyanobacteria dominance that corresponded to more stable, warmer, and nutrient-limited water conditions. Associated toxins were present and varied over time and space. Further research on the phytoplankton assemblages on the lake is needed in subsequent, non-Spillway opening years to evaluate the impact of river water pulses on the development of these toxic cyanobacterial blooms.

Keywords River diversions · Lake Pontchartrain · Mississippi River · Nutrient enrichment · Cyanobacterial blooms · Microcystin

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S. Bargu (✉) · J. R. White · C. Li · J. Czubakowski
Department of Oceanography and Coastal Sciences, School of the Coast and Environment, Louisiana State University, Baton Rouge, LA 70803, USA
e-mail: sbargu@lsu.edu

R. W. Fulweiler
Department of Earth Sciences, Boston University, Boston, MA 02215, USA

C. Li
Marine Ecosystem and Environment Laboratory, College of Marine Sciences, Shanghai Ocean University, Shanghai 201306, China

Introduction

Estuaries are transition zones between river and ocean environments and are subject to both marine influences (tides, waves, and the influx of saline water) and riverine influences (flows of nutrient rich freshwater and suspended sediment) (Li et al., 2009).

These transition zones are among the most biologically productive water bodies in the world (McLusky & Elliott, 2004). Estuaries provide habitat, refuge, nursery, and foraging areas to many higher trophic levels. Phytoplankton are considered to be one of the major primary producers in estuaries that support the diversity and productivity. As estuaries become increasingly subject to higher loads of bioavailable nutrients, eutrophic conditions become more pronounced shown by high algal production, hypoxia, and associated fish kills.

Water control structures, such as levees are designed to protect cities from flooding in the flood season due to increased upstream drainage area snowmelt. Along the lower Mississippi River, there are three river diversions with control structures, from which river water can be diverted into wetland or coastal water to reduce the pressure of the high water (White et al., 2009). Freshwater diversions also reduce salinity in affected estuaries and promote wetland productivity (Delaune et al., 2008; Gardner & White, 2010). As with any pulse of nutrients, there is the potential for algal blooms, including toxic ones, as well as hypoxia in the receiving basin.

The city of New Orleans, Louisiana, USA, is vulnerable to flooding events from the Mississippi River during high river stage. To prevent this, the Bonnet Carré Spillway was built to provide relief from floodwaters, with a diversion capacity of up to 7080 $m^3 s^{-1}$ of water. The Spillway was constructed in 1931 after the 1927 flood to protect the city of New Orleans from the Mississippi River water by diverting floodwater from the river into an oligohaline estuarine, Lake Pontchartrain. Since then, the Spillway has been opened for flood control on nine occasions, most recently in 2008 (White et al., 2009). After the 1997 Bonnet Carré Spillway opening, it was found that cyanobacterial blooms occurred in Lake Pontchartrain (Dortch & Achee, 1998; Turner et al., 1999). Nutrient introduction from the 1997 Bonnet Carré Spillway diversion (Day et al., 1998) was hypothesized to have resulted in the toxic cyanobacterial blooms of *Anabaena* and *Microcystis* (Dortch & Achee, 1998; Poirrier & King, 1998), considering the absence of high turbidity. Hepatotoxic microcystins from the cyanobacteria were present at that time (Dortch & Achee, 1998) and fish kills occurred (Poirrier & King, 1998).

During the most recent opening in 2008, the Bonnet Carré Spillway diverted up to 4785 $m^3 s^{-1}$ of the river water into Lake Pontchartrain with the total amount exceeding the lake volume, along with high concentrations of nitrate, dissolved reactive phosphorus (DRP), and dissolved silica (Si) concentrations. The total loading of nitrate alone was ~10,000 metric tons of NO_3–N (White et al., 2009).

The goals of this study were to evaluate nutrient enrichment and consequent response in phytoplankton biomass, including the toxic species in Lake Pontchartrain during and after the Bonne Carré Spillway opening in 2008. After several previous openings, it had been hypothesized that nutrient loading to the lake had triggered algal blooms. However, this assertion has never been investigated. This study included 10 stations covering the area from the diversion input to the middle of the lake spanning 30 km in the western half of the lake. This design allowed us to (1) characterize and define the extent of the river plume within the lake water, (2) monitor the collapse of the plume and subsequent mixing dynamics post-diversion, and (3) examine the response of phytoplankton biomass and community composition to environmental parameters influenced by the river water entering the lake.

Materials and methods

Study area

Lake Pontchartrain (Fig. 1) is a large (1630 km^2), oval-shaped, coastal lake system. The long axis of about 66 km is in the east–west direction while the short axis approximately 40 km is in the north–south direction. The lake is connected to the coastal ocean through three narrow tidal channels, i.e., the Rigolets, Chef Channel, and Industrial Canal with a mean depth of about 3.7 m (Fig. 1). The lake is located just north of New Orleans, LA and has an approximate volume of 6 km^3 and a residence time of ~57 days (Turner et al., 2004). Salinity in the lake varies depending on tidal flushing, river discharge, and wind conditions, ranging from 2 to 9 psu (practical salinity units), with higher values in the central and southern areas (Li et al., 2008). The Bonnet Carré Spillway provides a temporary connection with the Mississippi River with control structures consisting of 350 "bays" which are normally closed. The control structure is a mechanically operated concrete weir

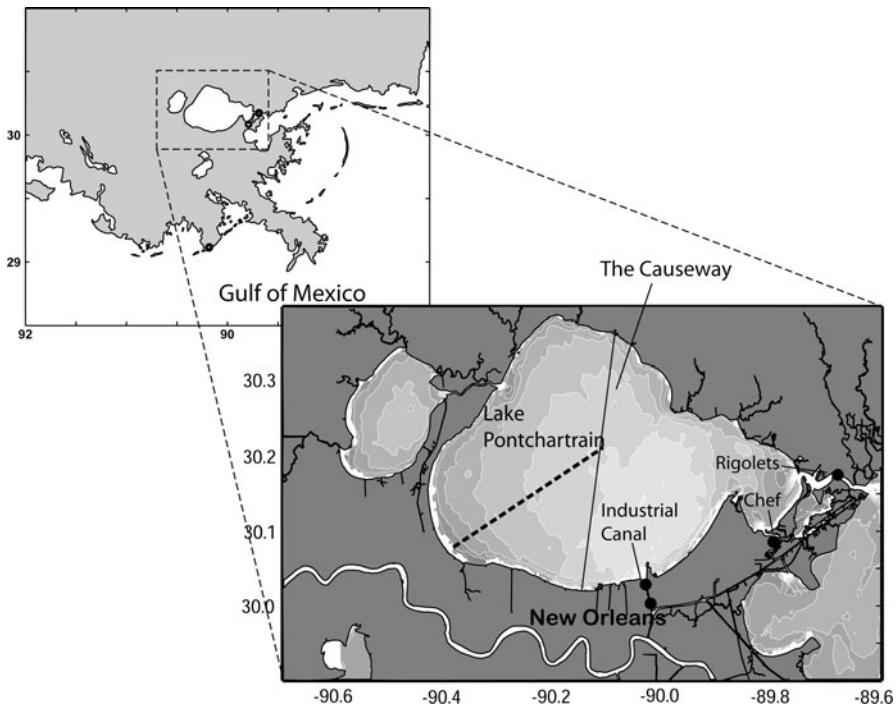

Fig. 1 Map of the Lake Pontchartrain showing the three outlets to the Gulf of Mexico (Industrial canal, Rigolets, and Chef Mansuer), the city of New Orleans, and the location of the Bonnet Carré Spillway, the Causeway, and the sampling transect (*dashed line*)

closed by 20 timbers in each bay. When all the bays are opened, it allows Mississippi River water into the Lake Pontchartrain at a rate of $\sim$7000 m^3 s^{-1}. The Bonnet Carré Spillway's control structure is along the north bank of the Mississippi River leading to a low-lying floodway of 2 miles wide and 5.7 miles in length connecting the Lake Pontchartrain. Guide levees define the boundaries of the floodway connecting the structure and the lake (Fig. 1).

Field sampling

A 10-station transect, approximately 30 km in length, was established spanning from the inflow point of the Bonnet Carré Spillway into Lake Pontchartrain trending in a northeasterly direction and terminating at the Lake Pontchartrain causeway (Fig. 1). This northeast–southwest oriented sampling transect includes stations from within the river water plume and within the lake water that was interacting with the plume, with the stations separated at 3 km intervals.

Water samples were collected at 10 cm below the surface at each station during the Spillway opening on April 28 and May 5, 2008, and after the Spillway closing on May 10, May 21, May 30, June 17, and July 22, 2008. Dissolved O_2, temperature, pH, and conductivity were measured with a hand-held YSI (Model 556) at 10 cm below the surface. Water samples were collected to determine the total suspended solids (TSS), nutrient concentrations, phytoplankton biomass and species composition, and levels of phycotoxin. For TSS and nutrients, 1-l samples were collected in acid-washed polyethylene bottles. An additional 1-l of water was collected for biological determinations. Approximately 250 ml of water were immediately preserved in a 2% gluteraldehyde solution for phytoplankton observations. The remaining samples were transferred on ice to Louisiana State University, Department of Oceanography and Coastal Sciences, for determination of cyanotoxin microcystins (MCs) concentrations. In the laboratory, fixed samples were kept in the dark, at room temperature until they were used while all other water samples were stored at 4°C.

Laboratory analyses

Nutrient and suspended solids analyses

Fifty milliliter of each water sample was vacuum filtered through 0.45 μm membrane filters upon return

to the laboratory and analyzed for DRP (Method 365.1; USEPA, 1993), NO_3-N (Method 353.2; USEPA, 1993), and NH_4-N (Method 350.1; USEPA, 1993) within 24 h on a Seal Analytical (Mequon, Wisconsin) AQ2+ discrete analyzer using standard colorimetric methods. Dissolved silica was measured on 0.45 μm membrane filtered subsamples using an autoanalyzer (Method 4500-SiO_2; APHA, 1989). For TSS, a measured volume of water was filtered through pre-ashed glass fiber filters (GFF Gelman), dried at 105°C and weighed to determine TSS.

Chlorophyll a (chl a) and microscopy analyses

One-liter surface water samples were collected per station for biological analyses in clean polypropylene bottles and stored on ice. Upon returning to the lab, chlorophyll *a* was determined for all stations as a measure of phytoplankton biomass. Twenty-five to 100 ml sub-samples of surface water were filtered through 25 mm GF/F filters. Filters were then extracted for 24 h in 90% aqueous acetone at −20°C and subsequently analyzed for chl *a* using a Turner fluorometer (Model 10-AU) (Parsons et al., 1984). Subsamples preserved with 2% glutaraldehyde and kept in a dark, at room temperature were used to determine the species composition of the phytoplankton community using an inverted microscope (Axiovert 135, Zeiss).

Phycotoxin measurements

Preliminary examination of the phytoplankton community revealed species of cyanobacteria capable of producing the cyanotoxin MCs were present in Lake Pontchartrain. Therefore, water samples were analyzed for MCs levels using Enzyme-Linked Immunosorbant Assay (ELISA) with a detection limit of 0.10 μg l^{-1} based on the most common variant, MC-LR, and its congeners.

To measure particulate MCs, an extraction process was formulated to allow detection of the cyanotoxin with purchased commercially available MC-ADDA ELISA kits (Abraxis, LLC, USA). A spike and recovery experiment following the extraction protocol produced extraction efficiencies of 93% for particulate MC. This involved adding MC-LR standard (Abraxis, LLC) directly to a clean GF/F filter to result in a final concentration of 7.5 μg l^{-1} in the extraction volume. For the particulate samples, the glass fiber filters from each replicate were allowed to reach room temperature and placed in a 15 ml round bottom glass centrifuge tubes with 5 ml of a 50% methanol, 1% acetic acid extraction solution. The filters and solution were vortexed (Fisher Scientific) for 1 min and sonicated using a Misonix Sonicator 3000, equipped with a microtip, for 2 min at 30–40 W. The solution was centrifuged for 10 min at 3000 RPM with a relative centrifugal force of 1,399*g* in an IEC Centra CL2 centrifuge (Thermo Electron Corp.). The supernatant was collected from the centrifuge tube and passed through a 0.20 μm syringe filter (Corning) with a surfactant free cellulose acetate (SFCA) membrane filter into a 7 ml scintillation vial. An additional 5 ml of extract solution was added to the already homogenized filter in each 15 ml centrifuge tube and the process of vortexing, sonicating and centrifuging was repeated. The resulting supernatant was filtered and added into the same scintillation vial used to collect the previous supernatant volume for a total extraction solution volume of up to 7 ml. All the processed samples were temporarily stored at 4°C until needed for analysis.

All samples were analyzed following the protocol included in the ELISA kit (Abraxis, LLC). The samples were analyzed in duplicate at a dilution of at least 1:10. Additionally, if samples analyzed were found to be greater than the range of the assay, the extract was diluted to 1:100 and reanalyzed. The absorbance data for each sample were collected using a micro-plate spectrophotometer set at a wavelength of 450 nm.

Data analyses

During the Spillway opening

In an effort to differentiate the diverted Mississippi river water from the lake water, we were able to use temperature, salinity, and nutrients to group the transect stations into two distinctly different categories: (1) within the plume and (2) outside the plume. There was very little variability for the first six stations within the freshwater plume while the seventh station was generally transitional and the remaining three stations were within the more brackish original lake water. For statistical treatment of the data, we combined the first six stations for both April 29 and

Table 1 Water quality parameters for the stations in Lake Ponchatrain for each of the period in 2008

	During Spillway opening (April 29, May 5)		Plume collapse period (May 10, May 21)		Post-plume period (May 31, June 17, July 22)	
	Within plume ($n = 12$)	Within lake ($n = 6$)	Within plume ($n = 14$)	Within lake ($n = 6$)	Within plume ($n = 14$)	Within lake ($n = 6$)
Temperature (°C)	19.3 ± 1.2	22.9 ± 1.0	26.0 ± 1.3	26.8 ± 0.7	30.4 ± 1.1	29.9 ± 1.3
Salinity (psu)	0.2 ± 0.0	2.9 ± 0.2	0.9 ± 0.6	2.6 ± 0.5	2.4 ± 0.9	2.1 ± 0.7
Dissolved O_2 (mg l^{-1})	8.9 ± 0.3	9.9 ± 0.3	9.6 ± 2.0	12.0 ± 1.4	9.6 ± 1.9	9.1 ± 0.4
pH (pH units)	7.7 ± 0.8	7.3 ± 1.4	8.0 ± 0.7	8.3 ± 0.3	8.3 ± 0.5	8.6 ± 0.1
TSS (mg l^{-1})	41.0 ± 14.6	22.7 ± 4.7	7.7 ± 3.0	16.9 ± 7.3	7.4 ± 2.4	10.5 ± 4.1
Nitrate (NO_3-N) (μM)	93.5 ± 5.3	19.4 ± 6.1	58.3 ± 32.3	4.8 ± 6.2	1.8 ± 0.7	2.5 ± 3.2
Ammonium (NH_4-N) (μM)	1.3 ± 0.8	2.2 ± 1.2	1.8 ± 1.1	1.0 ± 0.2	1.1 ± 0.8	1.0 ± 0.3
DRP (μM)	1.6 ± 0.3	0.3 ± 0.2	1.1 ± 0.7	0.3 ± 0.2	0.4 ± 0.3	0.6 ± 0.5
Silica (μM)	92.2 ± 9.8	28.7 ± 12.6	40.0 ± 41	7.6 ± 3.6	38.6 ± 37.9	32.1 ± 37.5
Chl *a* (μg l^{-1})	6.5 ± 4.0	29.2 ± 1.8	14.4 ± 8.6	15.6 ± 12.7	21.9 ± 13.4	25.5 ± 8.6

Data are mean ± standard deviation. Samples collected at 10 cm below the surface

May 5 samplings ($n = 12$) to represent the Mississippi River plume while grouping the three furthest stations over the two dates to represent the lake water (outside of the direct influence of the plume; $n = 6$).

After the Spillway closing

The sampling periods of May 10 and May 21 were grouped together as the "plume collapse" period. Similar to the Spillway opening period, stations were grouped as the plume stations (this time transitional seventh station was included as one of the plume stations) ($n = 14$) and the lake stations ($n = 6$). The following sampling dates of May 30, June 17, and July 22 were defined as the "normal period", as there was no longer any physical or chemical evidence of the river plume and therefore the stations were treated all as lake stations ($n = 20$). In order to demonstrate this, we grouped the "normal period" in Table 1 into stations of former plume (1–7) and lake (8–10) which clearly demonstrate there is no longer any difference between them.

Data were statistically compared using a one way ANOVA at an alpha of 0.05 and Student's *t* test. The interrelationship of measured parameters was investigated using Pearson product moment correlation analysis.

Results

During the Spillway opening (April 29 and May 5, 2008)

Between April 29 and May 5, 2008, Bonnet Carré Spillway was opened, influencing part of the lake by the river water intrusion. The results from these two sampling dates previously were reported in White et al. (2009). In summary, the plume water was colder and fresher than the lake water ($P < 0.001$), but both regions were well oxygenated and well mixed, with higher levels of TSS in stations located in the plume water ($P < 0.001$) (Table 1; Fig. 2a, b for salinity). High dissolved inorganic nitrogen, phosphorus, and silica levels were also detected in plume water (Figs. 3a, b, f, g, and 4a, b). Although the available nutrient levels were higher in the plume compared to lake stations on both April 29 and May 5 of 2008 ($P < 0.001$), plume stations contained lower algal biomass indicated by chl *a* values ($P < 0.001$). The mean chl *a* concentrations within the plume was 6.52 ± 4.01 μg l^{-1} over both sampling dates during the Spillway opening while the concentrations of chl *a* were significantly higher (29.2 ± 1.80 μg l^{-1}) for the three lake stations during the same time period (Fig. 4f, g; Table 1).

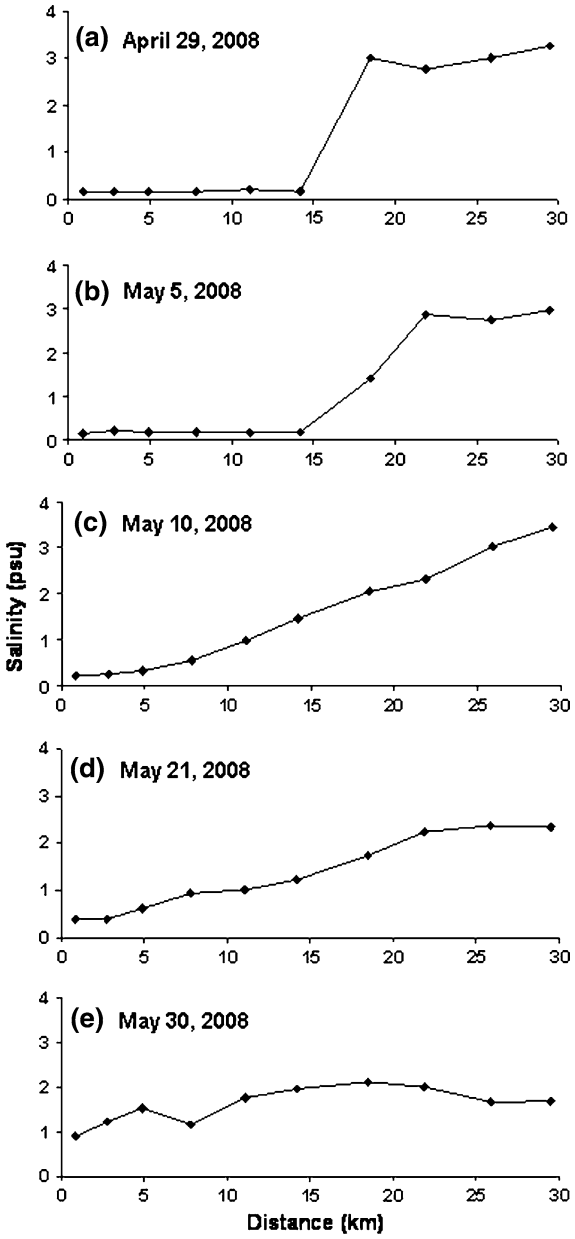

Fig. 2 Salinity (psu) measurements for stations along a transect running from within the surface water Mississippi River plume across a transitional station to lake water stations between April 29 and May 30, 2008. **a** April 29, **b** May 5, **c** May 10, **d** May 21, and **e** May 30

On both dates, samples from inside of the plume contained low phytoplankton biomass but showed higher diversity compared to the samples from outside the plume. Most dominant groups observed at the plume stations were centric diatoms—specifically chains of *Melosira*, and chlorophyte—mainly chains of *Klebsormidium*, while cyanobacteria, pennate diatoms, and small flagella were also present. On the other hand, lake stations were dominated by centric diatom chains of *Skeletonema*, and contained less of other groups including chlorophyte, cyanobacteria, pennate diatoms, and small flagellates.

Plume collapse period (May 10 and May 21, 2008)

After the Spillway was closed on May 8, 2008, gradual mixing between the two water bodies was observed throughout the month, indicated by the changes in salinity ($P < 0.05$) (Fig. 2c, d). Water temperatures were higher than the previous period ($P < 0.001$) and were no longer different between the plume and lake stations, averaging 26.2 ± 1.2°C. Overall, salinity at the plume stations increased but still remained fresher (0.86 ± 0.58 psu) compared to lake stations (2.5 ± 0.56 psu). Surface dissolved oxygen remained high for all the stations on May 10 (10.3 ± 2.1 mg l^{-1}). Dissolved oxygen data were not available for May 21. pH levels increased over time ($P < 0.05$) and were variable among the stations (Table 1).

Similar to the Spillway opening period, the dissolved inorganic N pool was dominated by nitrate during the plume collapse period and dropped significantly over time at the plume stations ($P < 0.05$) but did not change significantly at the lake stations (Fig. 3c, d). Both on May 10 and May 21, the nitrate surface concentrations were significantly higher at the plume stations (79.5 ± 26 μM and 37.2 ± 23.5 μM, respectively) compared to lake stations (7.4 ± 8.7 μM and 2.3 ± 0.08 μM, respectively) ($P < 0.001$). Ammonium concentrations were low (Table 1).

Dissolved reactive phosphorus was also higher within the plume stations on May 10 and May 21 with an average concentration of 1.1 ± 0.7 μM, while the mean lake water concentration was more than three times lower at 0.3 ± 0.2 μM (Fig. 3h, i) ($P < 0.05$). The dissolved silica concentrations were significantly higher on May 10 within the plume (75.4 ± 26.6 μM) when compared with the lake stations (7.1 ± 4.3 μM) ($P < 0.05$) (Fig. 4c, d). However, the concentrations significantly decreased at the plume stations on May 21 (4.6 ± 1.6 μM) ($P < 0.001$), while there were no significant change at the lake stations (8.1 ± 3.6 μM) (Table 1).

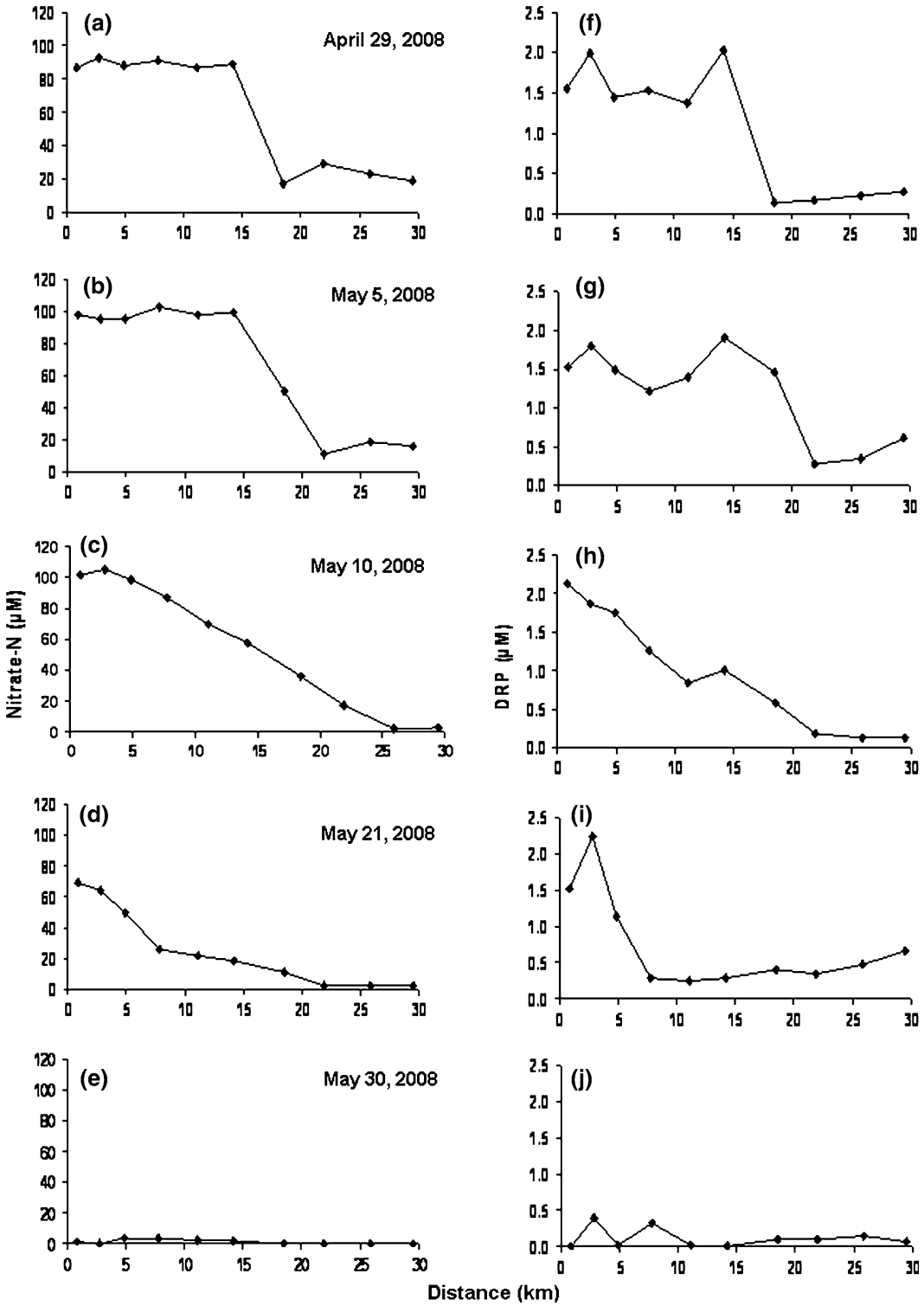

Fig. 3 Dissolved inorganic **a**–**e** nitrogen and **f**–**j** dissolved reactive phosphorus (DRP) concentrations (μM) along a transect running from within the surface water Mississippi River plume across a transitional station to lake water stations between April 29 and May 30, 2008. **a**, **f** April 29; **b**, **g** May 5; **c**, **h** May 10; **d**, **i** May 21; and **e**, **j** May 30

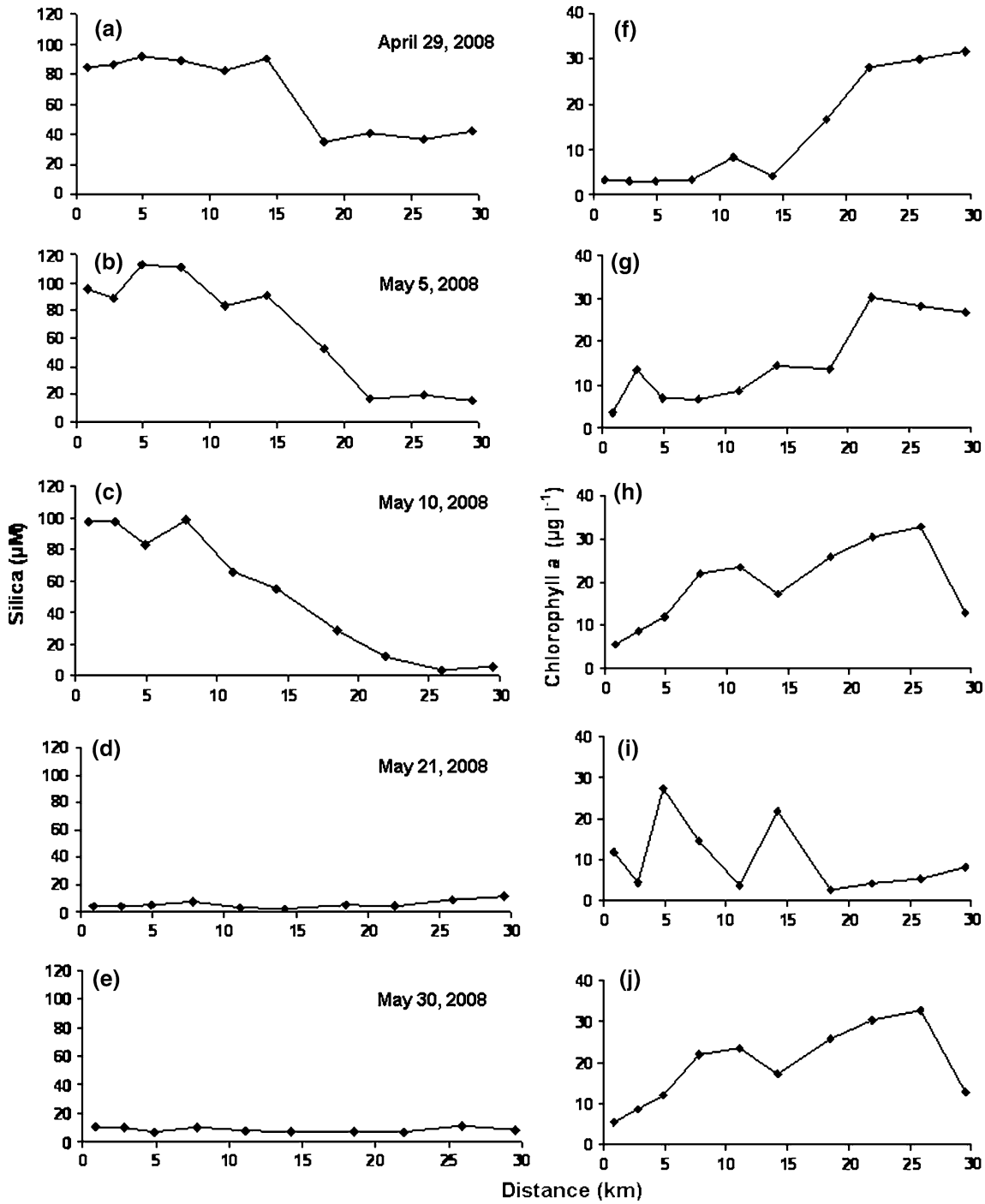

Fig. 4 Dissolved inorganic **a**–**e** silica (μM) and **f**–**j** chlorophyll *a* (μg l^{-1}) concentrations along a transect running from within the surface water Mississippi River plume across a transitional station to lake water stations between April 29 and May 30, 2008. **a**, **f** April 29; **b**, **g** May 5; **c**, **h** May 10; **d**, **i** May 21; and **e**, **j** May 30

Phytoplankton biomass fluctuated among the stations during the collapse period, with an increasing trend over time in plume stations (Fig. 4h, i). On May 10, chl *a* remained low at the first two plume stations (7.2 ± 2.3 μg l^{-1}), but increased at all the other plume stations compared to previous period

(20.1 ± 5.5 μg l^{-1}) ($P < 0.05$). On the same date, lake chl *a* levels were not significantly different from the previous period except for the station furthest from the diversion, T19—which dropped from 26.8 to 12.8 μg l^{-1}. Biomass varied among the stations but overall remained high on May 21 in plume stations (12.4 ± 9.5 μg l^{-1}), and a significant decrease was observed in lake stations (5.9 ± 2 μg l^{-1}) ($P < 0.05$) (Table 1; Fig. 4h, i).

Like the Spillway opening period, samples from inside of the plume water contained higher diversity compare to the samples from outside the plume. On May 10, the most dominant groups observed at the plume stations were still centric diatoms, dominated by *Melosira*, and chlorophyte *Klebsormidium*, while cyanobacteria, pennate diatoms, small flagellates, and dinoflagellates were also present. The lake stations continued to be dominated by centric diatom chains of *Skeletonema*, and chlorophyte was also present. On May 21, centric diatoms of *Melosira* and *Skeletonema* continued to be dominant in plume and lake stations, respectively, but other groups were replaced with toxic cyanobacteria species of *Microcystis* and *Anabaena* at low abundance. *Anabaena* was present only in the two stations furthest from the diversion.

Normal period (post-Spillway opening) (May 30, June 17 and July 22, 2008)

Both temperature and salinity levels increased over time during this period (Fig. 2e for May 30) and they were similar at all stations for each sampling date (2.3 ± 0.83 psu and 30.3 ± 1.2°C), while both dissolved oxygen and water pH remained similar compared to values from the collapse period (9.5 ± 1.6 mg DO l^{-1} and pH of 8.4 ± 0.4) (Table 1).

Dissolved inorganic nitrogen was mostly below the detection limit (1.4 μM) or low for all the sampling dates during the normal period (Fig. 3e for May 30). Phosphorus increased slightly since May 30—and remained similar for both June 17 and July 22. Silica levels were higher compare to May 21 ($P < 0.05$) and remained similar on May 30 and June 17 (11 ± 5.6 μM) but increased significantly at all stations on July 22 (87.9 ± 5.1 μM) ($P < 0.001$) (Table 1).

Phytoplankton biomass increased significantly at all stations on May 30 compared to the previous sampling date of May 21 ($P < 0.05$), while chl *a* levels were higher at the stations closest to the diversion (Fig. 4j). Chl *a* levels dropped on June 17 at the first eight stations (8.8 ± 1.7 μg l^{-1}) ($P < 0.001$), but were still high at the last two lake stations (31.6 ± 10.7 μg l^{-1}). Stations with low chl *a* levels on June 17 had significantly higher biomass on July 22 (28.7 ± 8.5 μg l^{-1}) ($P < 0.001$), while the last two stations remained similar to previous values (29.4 ± 1.1 μg l^{-1}).

On May 30, 2008, there was a visible shift from centric diatoms to potentially toxic cyanobacteria. Although still present, centric diatoms with species similar to previous samplings dates were approximately one order of magnitude lower. Potentially toxic cyanobacteria species, on the other hand, became commonly observed; *Anabaena* was the dominant species at the lake stations on May 30 and June 17. On June 17, *Skelotonema* was the only present diatom species at all stations with low abundance. Previously observed diatom, *Melosira* in plume stations was replaced with *Skelotonema*. On July 22, centric diatoms were replaced with pennate diatoms with low abundance and potentially toxic cyanobacteria were dominant at all stations, including *Anabaena, Raphidiopsis,* and *Cylindrospermopsis* spp.

Phycotoxin analyses

MC-producing species of *Anabaena* and *Microcystis* were observed both in the plume and lake stations at varying levels, with higher levels observed after the Spillway was closed. Corresponding concentrations of particulate MCs ranged from below detection (0.10 μg l^{-1}) to 1.7 μg l^{-1}, with the highest concentrations of MCs measured on May 21 and May 30, 2008 (Fig. 5). Variation in the concentrations of MCs corresponded to the patterns of cyanobacterial abundance. However, on July 22, a mix of potentially toxic cyanobacteria species capable of producing other type of toxins were dominant at all stations, including *Anabaena, Raphidiopsis,* and *Cylindrospermopsis* spp. and corresponding MCs levels were lower than the previous sampling dates (0.03 ± 0.02 μg MC l^{-1}) (Fig. 5).

Discussion

Fresh water intrusion from Mississippi River affected phytoplankton biomass and species composition due

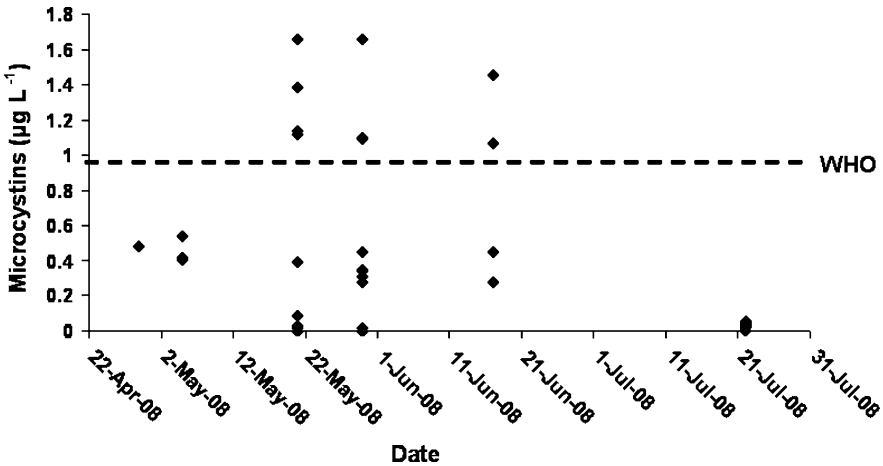

Fig. 5 Concentration of microcystins (µg MC l^{-1}) ($n = 19$) in surface water of sampling stations. *Dashed horizontal line* represents World Health Organization (WHO) advisory limit for tolerable daily intake (TDI) of 1.0 µg MC l^{-1}

to the increase of nutrient concentrations and by creating a fresher and more turbid environment. During the time of the diversion, the river plume did not mixed with the lake water effectively. However, after the Spillway was closed, the plume and lake water gradually mixed as indicated by salinity. The river pulse caused the lake nitrate and DRP concentrations to increase by more than five times in the plume stations, and nutrients decreased rapidly after the closure of the Spillway (plume collapse period). As the two previously published studies by Mize & Demcheck (2009) and White et al. (2009) have suggested, when riverine water entered into Lake Pontchartrain, diatoms and chlorophytes dominated the system. However, we found that after the Spillway's closure, over time there was a shift from diatom dominance to toxic cyanobacterial dominance that corresponded to a more stable, warmer and nutrient-limited condition. Associated toxin production was present most of the time but varied over time and among the stations.

During the Spillway opening, dissolved inorganic nutrient levels were very high in the plume water but with low phytoplankton biomass. The low biomass was likely due to light limitation related to high TSS and less stable water column. Dominance of diatom species between the plume and lake waters was different. While plume stations were primarily dominated by freshwater species of centric diatoms, stations outside of the plume were dominated by brackish species of centric diatoms. These differences support the hypothesis that exchange between phytoplankton communities was limited between the plume and lake waters for that period. Low levels of MCs were present in both plume and lake water during this period but we generally did not find corresponding MC-producing species. There are several potential reasons for a lack of MC-producing species observed in samples that had a detectable amount of MCs. Either an insufficient amount of sample was examined to observe large colonies of these species or physical disruption caused colonial MC-producing genera, such as *Microcystis,* to break apart. The latter is more likely since the majority of cyanobacteria cells observed were small (~2 µm in diameter), singular, coccoid cells. These cells may have been part of colonies that were disrupted by turbulence in the natural environment or homogenization of the sample water after collection for processing and examination on a Sedgewick Rafter slide. Therefore, these small singular cells were likely the source of the detected particulate and dissolved MC concentrations. Examining these genera, such as *Microcystis*, in colonies is a common practice and positive identification of singular cells from these colonies in natural phytoplankton assemblages is difficult.

After the Spillway was closed, plume and lake water started to gradually mix, generating the plume collapse period characterized by decreased TSS and nutrients. On May 10, chl *a* levels remained low at the first two stations, while the levels increased at all the other stations. Persistent turbid waters might have been suppressing the biomass at those first two stations, and a high nutrient pool together with lower TSS in the other plume stations were most likely the reasons for the higher biomass. Over time, both N and

Table 2 Pearson product moment correlation coefficients for water quality parameters for Lake Pontchartrain for the plume collapse period

	Distance	Temperature	Salinity	Nitrate	DRP	Silica
Temperature	0.09					
Salinity	0.86	0.33				
Nitrate	−0.58	−0.81	−0.74			
DRP	−0.54	−0.76	−0.70	0.91		
Silica	−0.37	−0.69	−0.52	0.84	0.66	
Chl *a*	−0.13	0.48	0.05	−0.29	−0.41	−0.15

Values are for $n = 30$, for $r > 0.56$; $P < 0.05$

P decreased significantly at all plume stations during the collapse period. The decrease in nutrients could generally be attributed to dilution or biological uptake. From the salinity distribution (Fig. 2), it could be seen that there was mixing with the more saline part of the lake during this time as the curve fluctuated, the low salinity water increased in salinity and the higher salinity water decreased. However, we did not see that same behavior in the DRP or NO_3 distribution which steadily decreased in the high concentration stations and did not increase at all in the stations furthest from the diversion. This observation along with a measured higher phytoplankton biomass over the same timeframe in the plume stations suggests the majority of the nutrient decrease during the plume collapse period was due to assimilation by the phytoplankton. Chl *a* concentrations were significantly negatively correlated with nitrate, dissolved silica, and phosphorus concentrations (Table 2) indicating that nutrients were depleted in phytoplankton uptake. Additionally, it is reasonable to assume that a portion of the nitrate could have been denitrified by microorganisms in the sediment (White & Reddy, 1999). The depletion of the bioavailable nutrients likely prevented further diatom growth and coincided with a shift from centric diatoms to toxic cyanobacteria dominance. This observation was also reflected with increases in dissolved silica levels throughout the transect, especially at the lake stations. The shift in dominance in May corresponded to increase in water temperature, decrease in nutrient availability and TSS that likely selectively supported cyanobacteria growth. Cyanobacteria have relatively slow growth rates (Robarts & Zohary, 1987) and require relatively warm conditions and long water residence times to proliferate (Jöhnk et al., 2008; Paerl & Huisman, 2008). They are also very competitive at low nutrient levels as opposed to diatoms—since some species of cyanobacteria can fix nitrogen and/or store phosphorus (Thompson et al., 1994; Dignum et al., 2005). Nitrogen-fixing, toxic cyanobacteria *Anabaena* spp. have been observed commonly at the lake stations during this time and correspond to higher toxin levels (up to 1.7 μg MCs l^{-1}) that were above the tolerable daily intake (TDI) guideline for microcystins in drinking water (1.0 μg MC l^{-1}) set by the World Health Organization (WHO).

At the end of May, plume and lake water were better mixed throughout the sampling transect. Temperature of the lake water was even higher and salinity returned to slightly higher, more normal levels. The MCs were still high on June 17, but the overall biomass was low likely due to the low levels of bioavailable nutrients. Nitrogen was mostly at below detection levels and phosphorus was also very low, while silica levels were increasing. In July, toxic *Anabaena* sp. was very common, but *Cylindrospermopsis and Raphidiopsis* species that can produce the toxin cylindrospermopsin (e.g., Saker & Griffiths, 2000; Li et al., 2002) were also commonly present—all are capable of fixing atmospheric nitrogen. During summer, N-limited conditions together with higher temperatures were most likely promoting the increase in these N-fixing species. Since MC—producing cyanobacteria species (*Microcystis* and *Anabaena*) were diluted with the other cyanobacteria species present (*Cylindrospermopsis and Raphidiopsis*), MCs levels were also much lower in July than the June levels.

There has been concern that diverting such a large nutrient load into the lake, albeit for flood control, could increase the potential for harmful algal bloom (HAB) forming phytoplankton species to proliferate (Turner et al., 1999, 2003). Even though river water introduced some freshwater diatom species into the lake during the opening, the diatoms did not dominate for long after the closing as the nutrient concentrations

decreased and the lake water started to mix with the plume water. However, the excess nutrients likely produced a diatom bloom right after the Spillway closing which stripped the water of bioavailable nutrients. HAB-forming cyanobacteria species became common in the lake when the nutrients were low. It is not clear whether the increasing presence of toxic cyanobacteria in the summer was due to the previous nutrient flux from the river input or it was a natural shift due to changing environmental conditions that were more favorable for cyanobacteria growth over diatoms. However, we observed that areas of the lake which saw little nutrient loading from the Spillway opening had some of the higher MCs levels (Bargu, unpublished). Since bioavailable nutrient concentrations returned to background levels prior to these increases, the nutrient loading event may not have been directly responsible for an increase in toxic cyanobacteria observed in this study.

River water discharge events, either pulsed at high levels or continuously at low levels, can potentially translate into enhanced primary production, phytoplankton community shifts, and bloom formation. This study found an increase in diatom abundance during the river pulse event, likely related to both the diatom influx in the river water and the more favorable conditions for fast-growing diatom population increase when compared to cyanobacteria. Only after the bioavailable nutrients (N, P, and Si) decreased to background levels did the toxin-producing species begin to proliferate. Further research is needed on more closely spaced spatial–temporal scales on non-diversion years to determine if there are any direct impact of river water nutrient input for the development of these toxic cyanobacterial blooms in the lake, especially as many of the species are N-fixers. Additionally, detection and monitoring of other type of cyanotoxins, including cylindrospermopsin, is needed in Lake Pontchartrain to evaluate the consequences for higher trophic level contamination, especially during the summer months.

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