Will Mississippi River diversions designed for coastal restoration cause harmful algal blooms?

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Abstract

Large-scale river diversions have been proposed as a way to transport sediment to the deteriorating Mississippi River deltaic plain. These diversions would also transport immense nutrient loads to shallow receiving estuaries, including open water areas where there may be potential for blooms of toxin-producing cyanobacteria. We examined environmental variables and cyanobacteria dynamics in the Lake Pontchartrain Estuary during the spring-summer period of two years: (a) 2011 when the system received a massive inflow of diverted Mississippi River water from the Bonnet Carré Spillway, and (b) 2012 when no diversion occurred. Cluster analysis of 180 samples collected over the course of both years identified five distinct water types grouped according to salinity, temperature, clarity, bioavailable nutrients, and chlorophyll *a*. Three of these water types were unique to the 2011 diversion event. We found no evidence of immediate, direct cyanobacteria bloom stimulation by diverted nutrients in the “high phytoplankton biomass” cluster associated with the 2011 diversion. Substantial nitrate assimilation by non-harmful phytoplankton occurred as the plume of diverted freshwater traveled through the system that year. The “summer” cluster characterized by nitrogen-limited conditions and including data from both years was characterized by significantly greater cyanobacteria abundance than all other clusters. There was no difference in cyanobacteria abundance between years within the summer cluster. Microcystin levels detected suggested low probability of acute health risks for recreational waters and were not correlated to cyanobacteria abundance. Site-specific understanding of the following is needed to guide diversion management: i) interannual phytoplankton community variability in non-diversion years, ii) cyanobacteria inputs from tributaries, iii) interactions between plume physics, chemistry, and phytoplankton dynamics, iv) internal phosphorus feedbacks that may indirectly link diversions to summer blooms, and v) controls on cyanotoxin production.

Keywords: Hydrologic alteration; River diversions; Nutrients; Cyanobacteria; Microcystin; Eutrophication

1. Introduction

Human modification of hydrology is widespread and impacts coastal zones around the world (Dynesius and Nilsson, 1994; Giosan et al., 2014). In estuaries, freshwater inflows have substantial influence on ecosystem function (Day et al., 2012). Altering these inflows, to enable navigation or to control floods, can modify physical features of an estuary (e.g., residence time,

vertical mixing, light attenuation, sediment transport, temperature, floodplain connectivity), as well as biogeochemistry (e.g., nutrient availability) (Vilhena et al., 2010; Day et al., 2009). These alterations can in turn influence biological communities of interest to ecosystem managers working to promote environmental quality and the maintenance of ecosystem services (Roelke and Pierce, 2011).

There is substantial interest in estuarine ecosystem response to episodic freshwater inflows due to both storm-driven runoff and hydrologic alteration (e.g., Roelke and Pierce, 2011; Paerl et al., 2014; Cook et al., 2010; Roy et al., 2013; Roelke and Spartharis, 2015a; Roelke and Spartharis, 2015b). This interest reflects the extensive influence of engineering projects on rivers and coasts, seasonal dynamics inherent to some systems, and increased variability in precipitation patterns between extreme drought and extreme flooding due to climate change (Dynesius and Nilsson, 1994; Paerl and Huisman, 2008; Roelke and Pierce, 2011; Wetz and Yoskowitz, 2013; Giosan et al., 2014). Past manipulations of waterways have degraded coastal environments and efforts are underway to revitalize these systems by restoring hydrologic connectivity (e.g., Kemp et al., 2014). It is critical to understand how abrupt changes in freshwater inflow and associated biogeochemistry – whether caused by stormflow dynamics, flood control structures, or restoration activities – impact phytoplankton communities at the base of estuarine food webs (Day et al., 2009; Roelke and Spartharis, 2015a).

The Mississippi River Delta provides a case study of coastal environmental degradation wrought in large part by efforts to support commerce and public safety through control of the river and its floodplain (Barry, 1997; Day et al., 2007; Kemp et al., 2014). The negative ecological consequences of upstream dams, downstream levees, and navigation canals on the delta have been described extensively (Swenson and Turner, 1987; Penland et al., 2000; Day et

al., 2007; Shaffer et al., 2009; Meade and Moody, 2010; Blum and Roberts, 2012; Kemp et al., 2014). Along with hydrologic alterations, the chemical composition of the Mississippi River has also undergone dramatic change in the past 50 years. Fertilization practices in the intensified agriculture of the Mississippi River basin have resulted in elevated concentrations of dissolved inorganic nutrients, especially nitrate-nitrogen (Goolsby and Battaglin, 2001; Turner and Rabalais, 2003). Therefore, transport of Mississippi River water inevitably involves the transport of immense loads of nutrients that can stimulate primary production in receiving coastal systems.

Presently there is great interest in using large-scale controlled river diversions to reconnect the Mississippi River to degraded deltaic landscapes, providing a way to transport sediment for land-building and slow rapid loss of coastal marshes (State of Louisiana, 2012; Wang et al., 2014; Kemp et al. 2014). It has been hypothesized that the addition of nutrient-rich waters via diversions may increase the frequency of blooms of toxin-producing cyanobacteria in receiving systems (Ren et al., 2009). Currently, there remains poor understanding of whether this is the case. Untangling the complexity of diversion-bloom links is imperative given the economic and cultural importance of coastal fisheries in Louisiana.

Here we examine phytoplankton community dynamics in a Louisiana estuary that is subjected to dramatic hydrologic alteration by a river diversion, and focus on potentially harmful cyanobacteria. The Lake Pontchartrain Estuary (LPE) in southeastern Louisiana, USA occasionally receives controlled inflows of Mississippi River water from the Bonnet Carré Spillway (BCS) (Figure 1). The BCS is used by the US Army Corps of Engineers to divert up to approximately 17% of the design flood stage Mississippi River into the LPE when the spring flood endangers the city of New Orleans. Openings, most recently in 1997, 2008, and 2011, vary in terms of both discharge and timing. Larger events can deliver more than twice as much

freshwater as the mean annual input from tributaries in less than 2 months (Roy et al., 2013). Recent research has suggested that large-scale diversions are required to achieve landscape-level restoration benefits in coastal Louisiana (Wang et al., 2014). The BCS is an appropriate existing analogue for such proposed diversions.

Previous studies have identified cyanobacteria blooms in Louisiana estuaries as a potential toxin contamination source (Table 1). Two cyanobacteria genera of local and further widespread concern are *Anabaena* and *Microcystis* (Dortch et al., 1999). Differences in the timing of occurrence between *Anabaena* and *Microcystis* are largely driven by nitrogen (N) biogeochemistry (Bouvy et al., 1999; Paerl et al., 2001; Paerl and Huisman, 2008). *Microcystis* spp. depend on dissolved inorganic N (DIN) in the water column and are typically observed in Louisiana estuaries in spring or early summer when DIN is provided by run-off or an episodic flood event. *Anabaena* spp., to the contrary, are able to fix atmospheric N, and can become the dominant species after water column DIN is depleted in later summer months and dissolved inorganic phosphorus (DIP) remains available (Bianchi and Argyrou, 1997; Garcia et al., 2010; Bargu et al., 2011; Riekenberg et al., 2014). Certain species of *Microcystis* and *Anabaena* can potentially produce anatoxins, microcystins, and saxitoxins (WHO, 1999). These toxins can cause neuro- and hepato-toxicity when ingested (Ibelings and Chorus, 2007; WHO, 1999), and some cyanobacterial substances may also cause less severe irritative health impacts (WHO, 1999). The LPE hosts commercial and recreational fisheries for multiple species, including blue crab (*Callinectes sapidus*), which has been found to be a potential vector of microcystins to humans in a nearby estuary (Garcia et al., 2010).

The BCS was most recently opened between May 9 and June 20, 2011 and the diverted freshwater plume influenced the majority of the LPE (Roy et al., 2013; Figure 1b). In a previous

study, we examined nutrient loading, freshwater inflow, nutrient dynamics, chlorophyll *a* dynamics, and limited observations of phytoplankton community composition to elucidate estuarine ecosystem response to the 2011 diversion (Roy et al., 2013). Here we build substantially upon that study using an expanded set of observations from 2011, as well as results from follow-up monitoring in 2012 when no BCS opening occurred. The specific objectives of this study were to: (1) utilize data collected in the LPE during both 2011 and 2012 to identify factors influencing cyanobacteria abundance, including the effects of hydrologic alteration, (2) further investigate nutrient uptake by phytoplankton and transport through the LPE during the 2011 diversion, (3) monitor microcystin concentrations in the LPE during 2011 and 2012 to determine potential for cyanobacteria-related health risks, and (4) determine the potential for northern tributaries to play a role in transporting cyanobacteria into the system.

2. Methods

2.1. Study Site

The LPE is a wind-dominated, shallow (mean depth = 3.7 m), 6 km³ estuary having a surface area of 1637 km² in southeast Louisiana. The estuary is oligohaline (salinity = 2-9; Li et al., 2008) and receives freshwater input from the fresher Lake Maurepas, several northern tributaries, and urban run-off from New Orleans (Figure 1) (Turner et al., 2002). Northern tributaries and Lake Maurepas are the dominant sources of freshwater during non-spillway years (McCorquodale et al., 2009). Water exiting Lake Pontchartrain through two eastern outlets eventually enters the Gulf of Mexico following passage through the estuarine Lake Borgne.

2.2. Bonnet Carré Spillway and northern tributary water discharge data

Water discharge data for the BCS in 2011 were provided by the US Army Corps of Engineers. Daily and monthly discharge data at five northern tributary monitoring stations during

2011 and 2012 were obtained from the USGS, and corrected to represent the entire watershed using methods outlined in Roblin (2008) and applied previously by Roy et al. (2013). The five tributary monitoring stations used were the Amite River near Denham Springs (USGS Station 7378500); the Tangipahoa River at Robert (USGS Station 7375500); the Tchefuncte River near Folsom (USGS Station 7375000); the Tickfaw River at Holden (USGS Station 7376000); and the Natalbany River at Baptist (USGS Station 7376500). Hydrologic data were investigated for February through August during both 2011 and 2012. This time period was selected based on the estuary's estimated mean residence time of 60 d (Swenson, 1980) and the months covered in this study's sampling regime (March through August). Observations for 2011 and 2012 were compared to 50-y monthly mean and median northern tributary flows (1962-2012).

2.3. Sample Collection

The sampling regime in 2011 involved multiple components. First, a 10-station, 30-km transect extending northeastwardly from the BCS inflow to the Lake Pontchartrain Causeway was monitored before, during, and after the 2011 BCS opening event (May-August, 12 sampling dates) (Figure 1a, T1, T11 to T19). Salinity, dissolved inorganic nutrients (nitrate+nitrite, NH_4^+ , DIP, and DSi), chl *a*, and Secchi depth data from this transect were previously reported in Roy et al. (2013). Limited phytoplankton cell abundances were reported for 3 of the 12 dates. Here, these same transect data are used as part of a multi-year cluster analysis. Additional samples were collected in 2011 at non-transect locations within the LPE and were used in this study. These included: (a) samples from northern stations collected on 4 dates during July-August in 2011 (N5 and N6 in Figure 1a); (b) BCS inflow (IN, Figure 1a) samples collected on the 12 dates that transect runs were made; (c) LPE outflow samples at Chef Menteur Pass (CMP, Figure 1a) collected on two days during the BCS opening and on an additional day soon after BCS closure;

and (d) additional samples on June 15 from stations (O1, O2, and O3, Figure 1a) lying between the transect stations and CMP as part of a lake-wide effort over two days that included sampling at the transect stations, IN, and CMP as well.

In 2012, three of the transect stations were again monitored during March-August (T1, T15, T19, Figure 1a). Also sampled in 2012 were four stations nearer to the northern shore (N1-N4, Figure 1a) and three stations within northern tributaries - Manchec Pass, the Tangipahoa River, and the Tchefuncte River (MAN, TAN, and TCH, Figure 1a).

In all cases, water samples were analyzed *in situ* at 10 cm below the surface for temperature, salinity, and dissolved oxygen (DO) using a handheld YSI (Model 556). YSI measurements were also taken in bottom waters at T1, T15, and T19 in 2011 and 2012. Water clarity was measured using a Secchi disc. Replicate water samples were collected in acid-washed polyethylene 1L bottles at 10 cm below the surface and placed on ice to be returned to the laboratory for analysis of chlorophyll *a* (chl *a*), microscopy, and dissolved inorganic nutrients. Phytoplankton cells were enumerated for all stations except O1, O2, O3, and CMP. For the June 15-16, 2011 sampling, mineral suspended solids were also measured. Microcystins were measured in samples collected from T1, T15, and T19 on all dates in 2011 and for all samples collected in 2012. Nutrient samples were also analyzed for bottom waters at T1, T15, and T19 in both 2011 and 2012. Results for bottom water samples are not reported here because there was no evidence of water column stratification at any time in 2011 or 2012 (i.e., no significant vertical heterogeneity in salinity, temperature, DO, or dissolved inorganic nutrients).

2.4. Nutrient and suspended solids analyses

In the laboratory, 30 mL of each water sample was vacuum-filtered through 0.45µm membrane filters and analyzed for nutrients. The N and P analyses were performed on a Seal

Analytical (Mequon, Wisconsin) AQ2+ discrete analyzer using standard colorimetric methods. Multiple forms of N were measured, including $\text{NH}_4\text{-N}$ according to EPA Method 350.1 (USEPA, 1993) and $\text{NO}_3^- + \text{NO}_2^-$ (abbreviated here as $\text{NO}_x\text{-N}$) according to EPA Method 353.2 (USEPA, 1993). Dissolved inorganic nitrogen (DIN) is the sum of $\text{NO}_x\text{-N}$ and $\text{NH}_4\text{-N}$. DIP was measured according to EPA Method 365.1 (USEPA, 1993). Dissolved Si (DSi) concentrations were measured on filtered subsamples using an O.I. Analytical Flow Solutions IV Autoanalyzer (Method 4500-SiO₂). Detection limits for $\text{NO}_x\text{-N}$, $\text{NH}_4\text{-N}$, DIP-P, and DSi were 0.016 mg N L⁻¹, 0.022 mg N L⁻¹, 0.003 mg P L⁻¹, and 0.002 mg Si L⁻¹, respectively. Any value found below these limits was assumed to equal the detection limit during data analyses. Nutrient limitation status was inferred using the DIN:DIP molar ratio, where ratios < 16:1 suggest N-limitation and those > 16:1 suggest P-limitation. Total suspended solids (TSS) was measured by filtering a measured volume of water through a pre-ashed glass fiber filter (GFF Gelman), dried at 105°C, and weighed (dry weight). These samples were then burned at 550°C to determine the organic suspended solids content as loss on ignition (ashed weight). Mineral suspended solids were then calculated as the difference between dry weight and ashed weight.

2.5. Chlorophyll *a* (chl *a*) and Microscopy Analyses

Chl *a* was measured in triplicate for all stations as a bulk measure of phytoplankton biomass. Fifty mL sub-samples of surface water were filtered through 25 mm GF/F filters (Whatman). Filters were then extracted for 24 h in 90% aqueous acetone at approximately -20 °C and subsequently analyzed for chl *a* using a Turner fluorometer (Model 10-AU) (Parsons et al., 1984). Additional whole water subsamples were preserved with 2% Lugol's solution and kept in a dark room at room temperature. A gridded Sedgwick-Rafter slide (Wildco) was used to examine duplicates of water from each Lugol's preserved archive sample on an inverted

microscope (Axiovert 135, Zeiss). In preparation for examination, each archive sample was uniformly mixed by inversion of the sample container for approximately thirty seconds before the subsample was loaded onto a Sedgwick-Rafter slide. The sample was then allowed to settle for 30-45 min before examination began. Cells were enumerated by individual or colony/chains and counts were grouped by taxon (centric diatoms, pennate diatoms, chlorophytes, cyanobacteria, other flagellates, and dinoflagellates). In order to determine abundance, the entire slide or a minimum of 150 individuals from the most dominant taxon were counted. These abundances were then averaged with their replicate to determine cells L^{-1} for each station and date. Most common species per taxon were measured and biovolume was estimated using published geometric models of phytoplankton (Sun and Liu, 2003). The average biovolume for each taxon was then multiplied by cell counts to obtain a representative biovolume. Average cell biovolumes recorded were 200, 78.5, 150, 4.2, 4, and 4188 μm^3 cell $^{-1}$ for centric diatoms, pennate diatoms, chlorophytes, cyanobacteria, other flagellates, and dinoflagellates, respectively.

2.6. Toxin extraction and analysis

Water samples were analyzed for both particulate and dissolved forms of the cyanobacterial toxin microcystin (MC). The samples were filtered through GF/F circles (Whatman) until clogged. The filtrates were collected for dissolved toxins, while the filters were kept for analysis of the particulate toxins. Both samples were kept at -20°C until processed.

An enzyme-linked immunosorbent assay (ELISA) kit (Abraxis LLC) was used to measure the MC equivalents in the two collected subsamples following the protocol in Garcia et al. (2010). ELISA is a competitive binding assay that is highly sensitive for MCs with a detection limit of 0.15 μg L^{-1} based on the most common variant, MC-LR, and its congeners. The cross-congener reactivity is high and has high sensitivity (Fischer et al., 2001). Each sample was run in

duplicate and at several dilutions in order to reduce interference from matrix effects. Absorbance was read at 450 nm using a micro-plate spectrophotometer. Non-detects were plotted at zero.

2.7. Data analysis

In total, there were 180 samples collected from within the LPE during 2011 and 2012 for which environmental parameters (salinity, water clarity, temperature, bioavailable nutrients), Chl *a*, and phytoplankton cell densities were all measured. Collectively, these samples capture temporal and spatial heterogeneity in the LPE with and without diversion influence.

The goal of statistical analysis here was to determine whether there are conditions in the LPE more favorable to cyanobacteria. This was accomplished using 5 steps. First, hierarchical cluster analysis using Ward's method with Euclidean distance measure was carried out to group all 180 samples into similar water types based on salinity, Secchi depth, water temperature, DIN, DIP, DSI, and Chl *a*. Z-scores were used to standardize data during clustering and the number of clusters was determined by identifying a greater than 10% change in the distance coefficient between steps. Cluster analysis has been applied in several previous studies of water quality (e.g., Boyer et al., 1997; Zeng and Rasmussen, 2005), including to group samples into distinct water types in the Mississippi River-influenced continental margin of the northern Gulf of Mexico (Chakraborty and Lohrenz, 2015). Second, after clusters had been defined, cyanobacteria cell density, cyanobacteria detection rate, and mean percentages of phytoplankton cell abundance for all phytoplankton groups were assessed for each water type. Third, data for all input variables in the cluster analysis and cyanobacteria cell density were plotted by water type using boxplots for inspection. Fourth, the nonparametric Kruskal-Wallis test was used to test the hypothesis that each parameter's distribution was the same across all water types. In cases where this hypothesis was rejected, post-hoc pairwise comparisons were made using the Dunn-Bonferroni approach (α

= 0.05). Finally, for the cluster identified as having the significantly greatest cyanobacteria abundance, nonparametric Mann-Whitney U tests ($\alpha = 0.05$) were performed to test the hypotheses that the distribution of cyanobacteria cell density in that group was the same across: (i) region (0-19 km vs. 22-39 km from BCS) and (ii) year (2011 vs. 2012). All statistics were performed using SPSS v.22.

3. Results

3.1. Characterization of freshwater inflows in 2011 and 2012

Freshwater input from northern tributaries to Lake Pontchartrain in 2011 was generally characterized by discharge rates $\ll 400 \text{ m}^3\text{s}^{-1}$ with the exception of one higher-discharge events due to precipitation in the watershed in early March (Figure 2a). Monthly discharge volumes from northern tributaries in 2011 were less than both 50-y mean and 50-y median volumes for all months between February and August except March (Figure 3). Freshwater input from tributaries in 2011 was supplemented by the relatively massive influx of Mississippi River water through the BCS, which had a maximum flow rate near $9000 \text{ m}^3\text{s}^{-1}$ (Figure 2a). The total volume discharged by the BCS during May and June of 2011 was 21.9 km^3 , or approximately 330% of the estuary's typical volume (Figure 3; Roy et al., 2013). The sediment-rich Mississippi River water plume traveled primarily through the southern half of the LPE (Figure 1b) and covered up to nearly 80% of the LPE surface area two weeks post-BCS opening (Roy et al., 2013).

In general, the LPE's northern watershed during the Feb-Aug period in 2012 was wetter than in 2011. February and March of 2012 were characterized by multiple flow events with discharges $> 400 \text{ m}^3\text{s}^{-1}$ (Figure 2b). For these two months, the volume discharged was greater than both 50-y mean and 50-y median volumes (Figure 3). However, as in 2011, monthly discharge volumes in April, May, and June of 2012 were below average (Figure 3). Discharges

from northern tributaries in July 2012 can be considered typical, while August flows were above average (Figure 3).

3.2. Distinct water types identified by the cluster analysis

The cluster analysis identified five distinct water types in the LPE during 2011 and 2012. Two of the water types included data from both years: *spring* (SP) and *summer* (SU). The remaining three clusters were unique to the 2011 diversion event: *light-limited* (LL), *high biomass* (HB), and *clear water* (CW). For 2011, the five water types can generally be interpreted to represent a temporal sequence starting with SP, followed by LL, HB, CW, and then SU (Table 2). However, on several of the sampling days, different stations were characterized by different water types due to spatial variation. Boxplots including outliers for all parameters across water types were examined to identify problematic classifications. Two were found: (1) a sample from May 28, 2011 was grouped in SP despite evidence of substantial diversion influence post-BCS opening ($\text{DIN} = 0.61 \text{ mg N L}^{-1}$, $\text{chl } a = 20.7 \text{ } \mu\text{g L}^{-1}$), and (2) a sample from August 22, 2012 was grouped into SP. In these two special cases, group membership was reclassified to be more logical, which did not result in changes in the conclusions from hypothesis testing. Statistical descriptions of the final five water types are shown in Figure 4.

3.2.1. The spring (SP) water type: characteristics and cyanobacteria abundance

The SP water type included samples from both 2011 and 2012 that were predominantly collected before the start of June ($n = 28$). Distinguishing features of SP included greater salinity (1 to 5), water temperature mostly near 25°C , N-limited conditions, and primarily low chl *a* (Figure 4). Cyanobacteria (*Microcystis*) were observed in 43% of SP samples and the maximum cyanobacteria cell density was $5.0 \times 10^5 \text{ cells L}^{-1}$ (Figures 4h and 4i). The phytoplankton community in SP was largely dominated by chlorophytes, dinoflagellates, centric diatoms, and

pennate diatoms (47%, 28%, 14%, and 10% of phytoplankton biovolume on average, respectively) (Figure 5). On average, cyanobacteria accounted for <1% of total phytoplankton biovolume in SP samples. In 2012, *Microcystis* spp. were initially found in the early spring at transect stations (1.4×10^5 cells L⁻¹).

3.2.2. The light-limited (LL) water type: characteristics and cyanobacteria abundance

All LL samples ($n = 32$) were collected between May 18 and June 25, 2011. Salinity throughout much of the LPE significantly plummeted as the plume of diverted Mississippi River water entered the system, marking a transition from SP to LL that year ($P < 0.05$, Figure 4a). Compared to SP, the LL water type was also characterized by significantly lower Secchi depth ($P < 0.05$, Figure 4b). No significant difference in water temperature between SP and LL was observed (Figure 4c). During LL, inorganic nutrient concentrations were elevated (Figure 4d-f), with DIN concentrations > 1 mg N L⁻¹, which was significantly greater ($P < 0.05$) than for all other water types (Figure 4d). At the same time, phytoplankton biomass measured as chl *a* remained low (≤ 8 µg L⁻¹ for most samples, Figure 4g). Cyanobacteria (*Microcystis*) were detected in 19% of LL samples (Figure 4i). Maximum cyanobacteria cell density observed in LL was 7.3×10^5 cells L⁻¹ (Figure 4h). Chlorophytes (including desmids, *Scenedesmus*, and *Klebsormidium*), diatoms (including *Melosira*), and dinoflagellates accounted for the majority of total phytoplankton biovolume during LL (Figure 5).

3.2.3. The high biomass (HB) water type: characteristics and cyanobacteria abundance

Observations spanning the west-east axis of Lake Pontchartrain on June 15-16, 2011 capture the transition between the LL plume phase and the high phytoplankton biomass (HB) phase that followed (Figure 6). As the plume travelled east during this time, mineral suspended solids largely settled out of the water column within the first 8 km and light penetration increased

(Figure 6b). The combined availabilities of light and nutrients enabled an increase in phytoplankton biomass (Figure 6d) and assimilation of DIP and DIN (Figure 6c). As is evident in the plume dynamics shown in Figure 6, this transition between LL and HB occurred rapidly. The water column in the HB plume phase was characterized by significantly greater salinity, Secchi depth, and temperature relative to LL ($P < 0.05$ in all cases; Figure 4a-c). Phytoplankton uptake of DIN and DIP was evident (Figure 4d-e) and mean chl $a \pm 1$ S.D. was $21 \pm 10 \mu\text{g L}^{-1}$, reaching up to $45 \mu\text{g L}^{-1}$, significantly greater than all other water types ($P < 0.05$ in all cases; Figure 4g). The detection rate for cyanobacteria during the HB plume phase increased to 50% (only *Microcystis* observed) (Figure 4i), and the maximum abundance of cyanobacteria observed was $4.5 \times 10^5 \text{ cells L}^{-1}$ (Figure 4h). Chlorophytes dominated the HB phase (mean proportion of total phytoplankton biovolume = 71%), while cyanobacteria accounted for <1% of biovolume on average (Figure 5).

3.2.4. The clear water (CW) water type: characteristics and cyanobacteria abundance

A distinct clear water (CW) plume phase followed the HB phase in July 2011 following BCS closure in late June (Table 2). The most distinguishing features of the CW phase were significantly greater water clarity than the SP, LL, and HB water types ($P < 0.05$ in all cases, Secchi depth up to 3.4 m), depleted DIN, a significant rebound in DIP concentrations relative to HB ($P < 0.05$), significantly depleted DSi, and a return to low phytoplankton biomass (chl $a \leq 10 \mu\text{g L}^{-1}$) (Figure 4). Despite the elevated phytoplankton productivity in the previous HB plume phase, dissolved oxygen was never observed to be below 5 mg L^{-1} within this data cluster, nor at any other time in 2011, including measurements of bottom waters at T1, T15, and T19. Cyanobacteria were observed in 18% of the samples collected for CW (*Microcystis*) (Figure 4i), and the maximum abundance of cyanobacteria was $5.4 \times 10^5 \text{ cells L}^{-1}$ (Figure 4h). Chlorophytes

and dinoflagellates dominated the phytoplankton community during CW (55% and 43% of biovolume on average) (Figure 5). Cyanobacteria accounted for 5% of phytoplankton cells and <1% of phytoplankton biovolume per sample on average in CW.

3.2.5. The summer (SU) water type: characteristics and cyanobacteria abundance

The final water type cluster identified was the summer (SU) water type including samples from both 2011 (August 10th) and 2012 (predominantly July and August) characterized by warm (near 30°C) and relatively clear waters having salinities similar to the SP cluster (Table 2, Figure 4a-c). N-limited conditions were pronounced in SU (Figure 4d) and phytoplankton biomass was low ($\text{chl } a \leq 9 \mu\text{g L}^{-1}$) (Figure 4g). The proportion of SU samples containing cyanobacteria (82%) was the greatest of any cluster (Figure 4h) and cyanobacteria abundance (maximum = $2.2 \times 10^6 \text{ cells L}^{-1}$) was significantly greater than in SP, LL, HB, and CW (adjusted $P < 0.05$ for all comparisons, Figure 4i). In SU, mean cyanobacteria cell abundance nearly matched that of chlorophytes (41% and 43% of total phytoplankton cells, respectively). However, the larger cells of chlorophytes accounted for far more of the total phytoplankton biovolume than did cyanobacteria (57% versus 3%) (Figure 5). Observed cyanobacteria in 2011 SU samples consisted of single cell or small groups of *Microcystis*. In 2012, *Microcystis* spp. were again observed in the SU samples and *Anabaena* spp. were abundant at both transect and northern stations in the early and late summer. Only *Microcystis* and *Anabaena* were observed throughout the entire sampling period - no other cyanobacteria were identified.

Considering all data in the SU cluster (Figure 7), cyanobacteria abundance tended to be greater at locations 22-39 km from the BCS ($n = 23$) than at stations 0-19 km from the BCS ($n = 16$), but there was variability in observations with distance ($P = 0.05$, Mann-Whitney U test).

There was no difference in cyanobacteria cell density between 2011 samples included in SU ($n = 13$) and 2012 samples included in SU ($n = 26$) ($P = 0.31$, Mann-Whitney U test).

3.3. Nutrient assimilation and transport to the LPE's eastern outlet during the 2011 diversion

Measurements made in this study capture nutrient uptake in the diverted freshwater plume in 2011, providing additional insight into drivers of phytoplankton dynamics and nutrient transport. DIP was depleted within 8-11 km from the BCS inflow during June 15-16, 2011 resulting in P-limitation of further primary productivity and near-conservative transport of the remaining DIN in the freshwater plume to the estuary's eastern outlet (Figure 6c, Table 3). Additional measurements made on June 8 also suggest that P-limitation resulting from the HB plume phase enabled a substantial portion of DIN loaded by the BCS to be exported from the estuary as nitrate+nitrite (Table 3). The mean outflow concentration of $\text{NO}_x\text{-N}$ on June 8 and June 15 at Chef Menteur Pass was 56% lower than the mean BCS inflow concentration for May 18, May 28, and June 4 (Table 3). $\text{NO}_x\text{-N}$ was again observed exiting the LPE on June 25 following BCS closure at a concentration 83% lower than the mean BCS inflow concentration for June 16 and June 21 (Table 3). Increased salinity, $\text{NH}_4\text{-N}$, and DIP at the Chef Menteur Pass on June 25 indicate that some dilution of the freshwater plume with estuarine waters and internal regeneration of nutrients had occurred by that time (Table 3).

3.4. Microcystins

Species of *Anabaena* and *Microcystis* capable of producing microcystins (MCs) were observed during 2011 and 2012. Observed MCs were largely in the dissolved form (Figure 8). Particulate MCs were only detected at low concentrations ($\leq 0.25 \mu\text{g L}^{-1}$) at one or more transect stations (T1, T15, and/or T19) in 2011 on May 28, June 16, and June 21. In 2012, particulate

MCs were never detected. In 2011 and 2012, total (dissolved + particulate) MC concentration was never $\geq 10 \mu\text{g L}^{-1}$, the concentration used to discern between “low” and “moderate” relative probability of acute health affects in recreational waters (WHO, 1999; Graham et al., 2009; Figure 8). The greatest total MC concentration observed in 2011 was $3.5 \mu\text{g L}^{-1}$ (Figure 8a). On March 28, 2012, total MCs were between 2.7 and $4.4 \mu\text{g L}^{-1}$ at all sites monitored. After that date, only one sample exceeded $1 \mu\text{g L}^{-1}$ for the remainder of the 2012 sampling period (T15 on April 25) (Figure 8b). Greater abundance of cyanobacteria did not correspond to greater MC concentration (Figure 8c).

3.5. Tributary observations

Observations in 2012 indicate that northern tributaries can serve as a source of both nutrients and cyanobacteria to the LPE (Table 4). N-limited conditions similar to those within the LPE in 2012 were observed in Pass Manchec for most sample days. In the Tangipahoa and Tchefuncte Rivers, DIN was detected at low concentrations ($< 0.2 \text{ mg N L}^{-1}$) throughout much of the spring and summer, while DIP and DSi concentrations ranged from 0.02 - 0.11 mg P L^{-1} and 2.6 - 6.4 mg Si L^{-1} . Phytoplankton biomass measured as chl *a* was variable at the tributary stations across both time periods, with concentrations as high as $58 \mu\text{g L}^{-1}$ in the summer. The greatest cyanobacteria cell density observed during this study (across both 2011 and 2012) was $2.9 \times 10^6 \text{ cells L}^{-1}$, measured in the Tangipahoa River on April 25, 2012. In 2012, *Anabaena* spp. were first present in low numbers only at tributary stations in late spring, followed by estuarine stations later in summer.

4. Discussion

4.1. Study limitations

Here, we have presented data from the LPE for spring-summer during two years, one with a BCS diversion event (2011) and another where no hydrologic manipulation occurred (2012). We do not presume that our observations during 2011 and 2012 can be extended to characterize all diversion and non-diversion years, respectively. BCS diversions vary in timing, duration, and volume, and phytoplankton dynamics have varied following recent diversions (Roy et al., 2013). Furthermore, seasonal phytoplankton trajectories in other warm-water systems have been shown to be variable (e.g., Roelke et al., 2004). Unfortunately, no long-term monitoring program currently exists in the LPE that targets water column physical, chemical, and biological parameters at the spatiotemporal resolution necessary to develop a detailed understanding of interannual and spatial variability. This hinders our capacity to determine: (i) the impacts of BCS diversions on the LPE system, and (ii) whether these impacts could be expected in other Louisiana estuaries that would receive inflows from large-scale diversions being planned. Despite these limitations, our observations from 2011 (the highest sampling resolution across space and time during a BCS event to date) and 2012, combined with observations made in past studies in coastal Louisiana (Table 1), contribute knowledge concerning when, where, and how cyanobacteria can influence the phytoplankton composition in river diversion-impacted open water estuarine environments.

4.2. Cyanobacteria dynamics

Potentially harmful cyanobacteria were observed in the LPE during spring and summer in both 2011 (*Microcystis*) and 2012 (*Microcystis* and *Anabaena*). During the BCS diversion in 2011, environmental conditions were highly dynamic, leading to multiple distinct water types. In studies of the Breton Sound Estuary, Day et al. (2009) and Riekenberg et al. (2014) both concluded that increased flushing by Mississippi River diversions during the warm season could

potentially help decrease toxin-producing cyanobacteria. Our results lend incremental support to this idea. We found no evidence that the diversion immediately and directly stimulated a bloom of toxic cyanobacteria. Cyanobacteria were outcompeted by chlorophytes, diatoms, and (on the basis of biovolume only) dinoflagellates during the light-limited and high biomass plume phases (Figures 4h and 5). Previous studies of the 1997 and 2008 BCS diversions also reported the absence of immediate stimulation of cyanobacteria (Turner et al., 2004; Mize and Demcheck, 2008; White et al., 2009; Bargu et al., 2011). Chlorophytes and/or diatoms appear to commonly outcompete cyanobacteria in turbulent, nutrient-rich diverted Mississippi River water (Table 1). Observations from outside of Louisiana provide further evidence that turbulence and water column mixing can favor phytoplankton groups other than cyanobacteria (e.g., Huisman et al., 2004). Cyanobacteria are highly buoyant phytoplankton and generally require stable water column conditions to maximize their light exposure (Walsby 1994; Kononen et al., 1996; Walsby et al., 1997; Merel et al., 2013). Additionally, the growth rate of cyanobacteria is typically lower than that of many algal species, including diatoms and chlorophytes (WHO, 1999). Short hydraulic residence times give faster growing phytoplankton species an advantage. The inability of cyanobacteria to capitalize more on available light and nutrients during the HB phase in 2011 may have been due to hydraulic flushing (Day et al., 2009; Mitrovic et al., 2011; Roy et al., 2013).

Our results and those of previous studies (Turner et al., 2004; Mize and Demcheck, 2008; Bargu et al., 2011; Table 1) suggest potential for cyanobacteria bloom formation in the summertime weeks and months following BCS closure. However, our findings here illustrate, for the first time, that such blooms may not necessarily be distinct from blooms in the summers of non-diversion years. Cyanobacteria cell density in the SU cluster containing samples from both

2011 and 2012 was significantly greater compared to all other clusters (Figure 4h), but no difference in 2011 vs. 2012 samples within the SU cluster was observed. In general, we found that cyanobacteria were most competitive in the LPE during 2011 and 2012 in samples characterized by increased water temperature, increased water clarity, and N-limited conditions (Figure 4).

The connection between the relatively high cyanobacteria abundance observed on August 10, 2011 and the BCS diversion that ended approximately 1.5 months earlier is difficult to ascertain. The initial dissolved inorganic nutrients loaded by the diversion had already been depleted ~1 month prior (Roy et al., 2013). *Microcystis* spp. are most commonly observed in fresher waters with salinity < 2 (Lehtimäki et al., 1997) and therefore might have benefited from the depressed salinity. However, *Microcystis* spp. can tolerate salinities up to 10 in some cases (Tonk et al., 2007) and *Anabaena* spp. have been found in waters with salinities as high as 15 (Moisander et al., 2002). Both of these upper limits extend beyond the common salinity range in the LPE (2-9; Li et al., 2008). We suspect that there may be an indirect effect of diversions on summertime cyanobacteria success via the internal phosphorus feedback from sediments. The rebound in DIP concentration in the LPE observed in the 2011 CW cluster was driven by internal P loading from sediments (Roy et al., 2012; Roy et al., 2013). Further research is needed to determine how river diversions affect P stocks within estuarine sediments and internal loading to the water column, where N-fixing cyanobacteria (*Anabaena* spp.) can benefit from increased P availability and N-limited conditions – perhaps even multiple years after the diversion event.

4.3. Potential role of tributaries

Tributaries function as more than “pipes” delivering nutrients to lake or estuary “reactors” (Carpenter et al., 1998); they also host biota that can likely influence dynamics within

receiving ecosystems (Conroy et al., 2014). Our results (Table 4) suggest that northern tributaries may play a role in transporting cyanobacteria into the LPE. Local environmental managers should therefore focus on improving poor nutrient use efficiency in agricultural and developed portions of the watershed (Roy et al., 2014). An unresolved question is whether or not elevated discharge from northern tributaries during late spring and summertime (greater than the largely below average discharges in 2011 and 2012; Figures 3 and 4) can promote cyanobacteria bloom formation in the LPE. Long-term monitoring of year-to-year variability in the phytoplankton community is required to answer this question.

4.4. Potential for harmful effects

Harmful effects of cyanobacteria in recreational waters can be divided into primarily irritative symptoms caused by unknown cyanobacterial substances (e.g., eye and ear irritation, skin rash, vomiting, diarrhoea, cold/flu symptoms, mouth ulcers, and fever), and the more severe hazard of exposure to high concentrations of known cyanotoxins, particularly microcystins (MCs) (Pilotto et al., 1997; WHO, 1999). The World Health Organization states that cyanobacteria abundance greater than 20×10^6 cells L^{-1} may result in relatively mild and/or low probabilities of adverse irritative health effects, while 100×10^6 cells L^{-1} is a guideline for moderate health alert in recreational waters (WHO, 1999). At the higher cell density, there is greater potential for scum formation where both local cell density and toxin concentration can increase by a factor of 1,000 or more in a few hours, leading to high risk conditions (WHO, 1999). The greatest cyanobacteria cell densities observed in the LPE during this study ranged up to 2.9×10^6 cells L^{-1} . Therefore, the risk of irritative symptoms was likely very low.

Microcystin concentrations observed also never indicated greater than low probability of acute health affects in the recreational waters of the LPE. Detection of relatively elevated toxin

concentrations did not correspond to greater cyanobacteria cell densities. Riekenberg et al. (2014) reported similar lack of correlation between MCs and potential MC producers in Breton Sound Estuary. Sinang et al. (2013) found weak cyanobacterial biomass-microcystin relationships across a suite of lakes, leading them to conclude that there is a high risk of error connected to indirect microcystins risk assessment from cyanobacterial biomass. This greatly complicates the management of toxic cyanobacteria blooms.

4.5. Nitrogen transformations in open water systems during diversions

Our results support the idea that partially enclosed open water estuarine environments receiving diverted Mississippi River water can serve to reduce DIN concentrations reaching the coastal ocean (Mitsch et al. 2001; Perez et al., 2011), but this reduction is limited by DIP availability (Figure 6, Table 3). Previous research has shown that flux of $\text{NO}_x\text{-N}$ into LPE sediments where denitrification can occur is a very minor loss pathway during BCS events due to the high loading rate and limited bed surface contact (Roy and White, 2012). Therefore, the decrease in water column DIN observed during the HB phase was predominantly driven by phytoplankton uptake (Figure 6), primarily that of chlorophytes (Figure 5, HB). Our observation of DIN transport across the system enabled by P-limitation (Figure 6) conforms to previous observations of the Atchafalaya and Mississippi River plumes (e.g., Lane et al., 2002; Sylvan et al., 2006; Perez et al., 2011). P-limitation thus serves to expand the spatial influence of nitrate+nitrite loaded by river diversions. Future studies of BCS diversions should focus on better quantifying the retention of nutrients in the LPE, including both dissolved and particulate species. Additionally, more work is needed to determine whether or not nutrient retention in the LPE has longer-term impacts on internal nutrient cycling (e.g., internal P loading from sediments) and blooms of toxin-producing cyanobacteria.

4. Conclusions

Our findings support the following conclusions:

- 1) The 2011 BCS diversion event did not result in immediate, direct stimulation of a harmful cyanobacteria bloom. Cyanobacteria were outcompeted primarily by chlorophytes during the period of nutrient assimilation and high phytoplankton biomass immediately following the diversion in 2011.
- 2) Cyanobacteria abundance was significantly greater during summertime N-limited conditions than for all other data clusters, with no significant difference found in the summer cluster between the diversion (2011) and non-diversion (2012) years.
- 3) Our findings lend support to the hypothesis that northern tributaries play a role in transporting cyanobacteria into the LPE. Further work is needed to clarify this role.
- 4) The greatest cyanobacteria cell densities observed did not indicate risk of irritative health effects. Additionally, microcystins were never detected at a level suggesting greater than low probability of acute health effects in recreational waters. Microcystins were not correlated to cyanobacteria abundance, complicating the distinction of a “harmful bloom” in the LPE.
- 5) Open water systems (like the LPE) receiving Mississippi River diversions could serve to reduce DIN loads to the coastal ocean via the mechanism of non-harmful phytoplankton assimilation, but, given the high DIN:DIP ratio of Mississippi River water, DIN uptake is ultimately limited by DIP availability.

In the past, LPE phytoplankton have been studied most intensively during river diversions or weather events (e.g., Turner et al., 2004; White et al., 2009; Mize and Demcheck 2009; Pinckney et al., 2009; Bargu et al., 2011; Roy et al., 2013). We stress that long-term studies (including

both diversion and non-diversion years) are needed to fully understand the effects of Mississippi River diversions on phytoplankton communities in open water estuarine environments. These studies should aim to: i) document seasonal and interannual variability in phytoplankton community composition during non-diversion years, ii) examine all potential pathways for nutrient and cyanobacteria transport into systems of interest, iii) investigate the interaction between diversion plume physics, chemistry, and phytoplankton dynamics, iv) document internal nutrient cycling pathways (e.g., internal phosphorus loading from sediments) that could potentially indirectly link diversions to harmful cyanobacteria blooms in months and years following diversions, and v) enhance understanding of controls on toxin-production by cyanobacteria. Lessons learned concerning river diversions in coastal Louisiana provide knowledge that will likely also be useful in other locations around the world where dramatic hydrologic alterations influence ecosystem function.

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Table 1. Prior observations of potentially harmful cyanobacteria in coastal Louisiana.

System	Observation	Citation
Lake Pontchartrain Estuary	Small surface accumulation of <i>Anabaena</i> sp. near the northern shore in June 1993, large surface accumulations of <i>Anabaena</i> spp. in late June 1994, and a bloom of <i>Anabaena</i> sp. from late June through mid-July in 1995.	Porrier and King (1998)
	Cyanobacteria observed making up 10-62% of total phytoplankton abundance during May 1995 to May 1996. Bloom of <i>Anabaena</i> sp. in July 1995 (10^6 cells L^{-1}) hypothesized to be influenced by persistent N-limited conditions in the water column.	Bianchi and Argyrou (1997)
	Dramatic phytoplankton bloom consisting of <i>Anabaena</i> spp. and <i>Microcystis</i> sp. during June 1997 (mean chl $a = 334 \mu g L^{-1}$, maximum chl $a = 855 \mu g L^{-1}$) nearly 2 months following the closure of the Bonnet Carré Spillway, which delivered $11.7 km^3$ of freshwater over ~1 month. Bloom hypothesized to be linked to diversion.	Turner et al. (2004)
	In 2008, <i>Anabaena</i> spp. first observed outside of Bonnet Carré Spillway plume influence (diversion open April 11 to May 8, delivered $7.5 km^3$ of freshwater). <i>Anabaena</i> spp. gained dominance on May 30 after mixing of	Bargu et al. (2011)

	diverted freshwater and estuarine water. Cyanobacterial dominance corresponded to a more stable, warmer, and N-limited water column. Concentrations of microcystins exceeded 1 µg L ⁻¹ on May 21, May 30, and June 17.	
	High cyanobacteria cell density observed during period of late April to late May 2008 when freshwater from the Bonnet Carré Spillway was mixed with estuarine water. Dominance of cyanobacteria in July and August 2008 during post-diversion period of low nutrient concentrations. Cyanobacteria accounted for >95% of phytoplankton community in August. Cell densities of <i>Anabaena</i> sp. and <i>Cylindrospermopsis raciborskii</i> reached up to 25 x 10 ⁷ cells L ⁻¹ and 2 x 10 ⁶ cells L ⁻¹ , respectively, during monitoring period.	Mize and Demcheck (2008)
Breton Sound Estuary	Cyanobacteria abundance usually highest in summer and fall when water temperatures were elevated. Absence to low presence of cyanobacteria during inflows of Mississippi River water from the Caernarvon diversion. Cyanobacterial chlorophyll was most closely related to phosphorus, light, and temperature. Hypothesized that diverted freshwater flushes cyanobacteria out of the system.	Day et al. (2009)
	Presence of both <i>Anabaena</i> sp. (up to 8.9 x 10 ⁴ cells L ⁻¹) and <i>Microcystis</i> sp. during summer and fall months. Microcystins detected (up to 2.92 µg MCs L ⁻¹) that pose potential risk to human health and economically important estuarine food webs. Warm, nutrient-limited water column is favorable for large cyanobacteria blooms. Cyanobacteria out-competed by chlorophytes during cooler periods with freshwater input from the Caernarvon diversion.	Riekenberg et al. (2014)
Barataria Estuary	<i>Anabaena</i> spp. observed to be the major cyanobacteria in all seasons in Lake Salvador (10 ⁴ to 10 ⁵ cells L ⁻¹) and Lac des Allemands (10 ⁶ to 10 ⁸ cells L ⁻¹), as well as during summer in Lake Cataouatche (10 ⁶ to 10 ⁷ cells L ⁻¹). <i>Microcystis</i> spp. have also been observed at high abundances during summer in Lac des Allemands (10 ⁷ to 10 ⁸ cells L ⁻¹) and at lower cell densities in the other two lakes (10 ⁵ to 10 ⁶ cells L ⁻¹). Suggested that additions of nutrient-rich waters via diversions of Mississippi River water might increase the frequency of algal blooms, including noxious and toxic cyanobacteria, based on bioassay experiments.	Ren et al. (2009)
	Blooms of <i>Microcystis</i> sp. (up to 2.4 x 10 ⁶ colonies L ⁻¹) and <i>Anabaena</i> sp. (up to 5 x 10 ⁶ cells L ⁻¹) in Lac des Allemands during spring and summer months, respectively. Microcystins detected in tissue of blue crabs.	Garcia et al. (2010)

Table 2. Distinct water types in the LPE during 2011 and 2012 identified by the cluster analysis (total $n = 180$). Note that several days in 2011 were characterized by spatial variation in water type, hence the overlapping date ranges. Stations listed are shown in Figure 1a.

Water type cluster	Temporal and spatial notes	n
Spring (SP)	Includes samples collected before or on May 18 during both years. Stations included in 2011: IN and transect stations (T1-T19). Stations included in 2012: T1, T15, T19, N1-N4.	28
Light-limited (LL)	Includes samples collected between May 18 and June 25, 2011 from IN and transect stations (T1-T19).	32

High Biomass (HB)	Includes samples collected between May 28 and June 30, 2011 from IN and transect stations (T1-T19).	42
Clear Water (CW)	Includes samples collected during July 2011 from IN, transect stations (T1-T19), N5, and N6.	39
Summer (SU)	Samples in 2011 collected during August. Samples in 2012 collected on or after May 15 (primarily July and August). Stations included in 2011: IN, transect stations (T1-T19), N5, and N6. Stations included in 2012: T1, T15, T19, N1-N4.	39

Table 3. Measured salinity and dissolved inorganic nutrient concentrations during the 2011 Bonnet Carré Spillway (BCS) event in Lake Pontchartrain at the BCS inflow (labeled “IN” in Figure 1a) and exiting the Chef Menteur Pass (labeled “CMP” in Figure 1a). BD = below detection.

Date	----- BCS Inflow -----				----- Chef Menteur Pass -----			
	Salinity	NO _x -N	NH ₄ -N (mg L ⁻¹)	DIP-P	Salinity	NO _x -N	NH ₄ -N (mg L ⁻¹)	DIP-P
18-May-11	0.14	1.11	0.038	0.054				
28-May-11	0.15	1.20	0.032	0.058				
4-Jun-11	0.17	1.08	BD	0.041				
8-Jun-11					0.28	0.70	BD	BD
15-Jun-11					0.21	0.29	0.031	BD

16-Jun-11	0.18	1.42	0.024	0.069				
21-Jun-11	0.20	1.37	BD	0.048				
25-Jun-11					0.72	0.24	0.118	0.072

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Table 4. Chemical and biological observations made in the northern tributaries during 2012. BD = below detection.

	Salinity	DIN (mg N L ⁻¹)	DIP (mg P L ⁻¹)	DSi (mg Si L ⁻¹)	Chl <i>a</i> (µg L ⁻¹)	Cyano- bacteria (10 ⁶ cells L ⁻¹)
Pass Manhec						
28-Mar-12	1.86	BD	0.04	2.73	4.7	0.25
25-Apr-12	0.54	0.11	0.09	3.45	4.5	0.00
15-May-12	1.60	BD	0.05	3.33	11.7	0.08
3-Jul-12	1.41	BD	0.06	2.77	20.9	0.58
1-Aug-12	0.74	BD	0.09	2.93	7.1	0.86
22-Aug-12	1.07	BD	0.06	3.33	7.3	0.21

**Tangipahoa
River**

28-Mar-12	0.20	0.07	0.05	3.71	7.2	0.25
25-Apr-12	0.07	0.16	0.03	5.73	15.6	2.90
15-May-12	0.19	0.11	0.02	6.40	9.6	1.43
3-Jul-12	0.34	BD	0.05	5.37	9.9	2.33
1-Aug-12	0.06	0.14	0.05	6.13	21.2	0.74
22-Aug-12	0.11	0.19	0.07	4.25	6.2	0.10

**Tchefuncte
River**

28-Mar-12	0.04	0.06	0.05	2.56	3.1	0.00
25-Apr-12	0.77	0.19	0.08	3.89	7.5	0.00
15-May-12	1.25	0.07	0.04	3.91	10.0	0.28
3-Jul-12	0.77	BD	0.09	3.05	47.4	0.96
1-Aug-12	0.08	BD	0.08	3.11	57.7	0.33
22-Aug-12	0.10	0.16	0.11	4.05	11.9	1.18

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Figure 1. (a) Map of the stations in Lake Pontchartrain sampled during 2011 and/or 2012 (see text). (b) MODIS 250m imagery of coastal Louisiana on May 17th during the 2011 Mississippi River Flood provided by the Earth Scan Lab at Louisiana State University. A plume of sediment-rich freshwater can be seen entering Lake Pontchartrain via the Bonnet Carré Spillway and traveling through the estuary from west to east.

Figure 2. Freshwater discharges ($\text{m}^3 \text{s}^{-1}$) from the Bonnet Carré Spillway (BCS) and the northern watershed in 2011 (a), and from the northern watershed in 2012 (b). BCS discharge data were provided by the US Army Corps of Engineers.

Figure 3. Monthly freshwater input volumes (km³) to Lake Pontchartrain from northern tributaries during Feb-Aug in 2011 and 2012. Also shown are the 2011 input volumes from the Bonnet Carré Spillway, as well as 50-y mean and median input volumes from northern tributaries (1962-2012).

Figure 4. Boxplots for water quality parameters (a-g) and cyanobacteria cell density (h) for the five water type clusters (see Table 2). $\circ$ denotes samples $> Q3 + 1.5 \times IQR$ or $< Q1 - 1.5 \times IQR$. Δ denotes samples $> Q3 + 3 \times IQR$. The rate of cyanobacteria detection for each cluster is also shown (i). Clusters include data from 2011 (Roy et al., 2013; this study) and 2012 (this study). Different letters within panels indicate statistically significant differences (adjusted $P < 0.05$, Dunn-Bonferroni).

Figure 5. Mean percent of (a) total cell abundance and (b) total biovolume accounted for by each individual phytoplankton group for each water type cluster.

Figure 6. Water quality parameters during the 2011 Bonnet Carré Spillway (BCS) event with linear distance from the BCS inflow on June 15 (distances of 30-61 km from the BCS, shown as O1-O3 and CMP in Figure 1a) and June 16 (distances of 1-19 km from the BCS, T1-T16 on transect in Figure 1a; Roy et al. 2013). LPC = Lake Pontchartrain Causeway. CMP = Chef Menteur Pass. Plume phases delineated here for the 7 transect stations are described in Table 2.

Figure 7. Cyanobacteria cell abundance for 2011 and 2012 LPE samples included in the *summer* (SU) cluster as a function of: (a) distance from the Bonnet Carré Spillway in the southwest corner of the estuary, and (b) day of the year. Northern and transect stations are labelled with “N” and “T”, respectively, in Figure 1a. See methods for a description of which stations were monitored each year. Dates are shown above data points.

Figure 8. MC (dissolved + particulate) concentrations in Lake Pontchartrain during 2011 (a) and 2012 (b). In (a) and (b), $\circ$ = transect stations T1, T15, and T19. Additionally, in (b), Δ = northern stations N1, N2, N3, and N4, and X = tributary stations MAN, TAN, and TCH. The shaded portion in (a) delineates the time during which the BCS was open in 2011. The relationship observed between MC and cyanobacteria cell abundance for all data in both years combined is shown in (c).