Influence of the Mississippi River on *Pseudo-nitzschia* spp. Abundance and Toxicity in Louisiana Coastal Waters

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Abstract

The presence of domoic acid (DA) toxin from multiple species of *Pseudo-nitzschia* is a concern in the highly productive food webs of the northern

Gulf of Mexico. We documented the *Pseudo-nitzschia* presence, abundance, blooms, and toxicity over 3 years along a transect ~100 km west of the Mississippi River Delta on the continental shelf. *Pseudo-nitzschia* were present throughout the year and occurred in high abundances (>10^4 cells l^{-1}) in the early spring months during high Mississippi River (MSR) flow (~20,000 m^3 s^{-1}) but were most abundant (>10^6 cells l^{-1}) when MSR discharge was relatively lower among the spring months. A high particulate toxin production (maximum reaching 13 µg DA l^{-1}) was associated with the high cell abundances and exceeded, by an order of magnitude, prior reports of particulate DA concentrations in Louisiana coastal waters. Differences in *Pseudo-nitzschia* peak times and its toxicity were correlated mainly with the timing and magnitude of MSR discharge and changes in associated parameters such as nutrient stoichiometry and salinity. A negative relationship between high MSR discharge and *Pseudo-nitzschia* and particulate DA concentrations was documented. These riverine dynamics have the potential to influence DA contamination in pelagic and benthic food webs in the coastal waters of the northern Gulf of Mexico.

Keywords

Harmful algal blooms
Pseudo-nitzschia
Domoic acid
Mississippi River
Louisiana coastal waters
Northern Gulf of Mexico

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Introduction

The continental shelf of the northern Gulf of Mexico is physically and biologically controlled by the variability of the Mississippi and Atchafalaya River discharges and their particulate and dissolved constituents, including high concentrations of dissolved inorganic nutrients, which influence phytoplankton biomass and primary production (Sklar and Turner 1981;

Lohrenz et al. 2008; Lehrter et al. 2009; Turner and Rabalais 2013). Some of the impacts from eutrophication, such as hypoxia and harmful algal blooms (HABs), have been the subject of interest for decades (Rabalais et al. 1996; Steidinger et al. 1998; Dortch et al. 2001; Parsons et al. 2002; Rabalais 2004; Rabalais et al. 2007; Parsons et al. 2013). The Mississippi–Atchafalaya River complex serves as a major conduit, transporting sediment and nutrients from a watershed encompassing 41 % of the contiguous USA (Turner and Rabalais 1994). The nitrogen concentrations in the Mississippi River have increased since the 1950s (Turner and Rabalais 1991; Turner and Rabalais 2003; Rabalais et al. 2010) primarily due to agricultural land use practices (Alexander et al. 2008). The extent and persistence of bottom water hypoxia on the Louisiana/Texas, and now Mississippi shelf, is a result of stratification (salinity and temperature) and high primary productivity due to these increased nutrient loads. The result is the largest human-caused coastal hypoxic zone in the USA and second largest worldwide, covering an expanse of up to 22,000 km^2 in mid-summer (Rabalais et al. 2007; Rabalais et al. 2010).

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These hypoxic zones are created by excessively large phytoplankton production. The phytoplankton community is commonly dominated by chain-forming diatoms, such as *Skeletonema*, *Chaetoceros*, and *Pseudo-nitzschia* in March to May and by picocyanobacteria during June to October (Dortch et al. 2001; Baustian et al. 2011; Schaeffer et al. 2012). The area is also subject to large blooms of potentially toxic phytoplankton, which are mainly the common spring diatom *Pseudo-nitzschia*, and occasionally the dinoflagellates *Karenia brevis*, *Alexandrium* spp., *Prorocentrum minimum*, and *Dinophysis caudata*, and the raphidophyte *Heterosigma akashiwo* (Dortch et al. 1997; Dortch et al 1998; Parson et al. 2015; Bargu and Rabalais et al. unpublished data). *K. brevis* is widely distributed in the northern Gulf of Mexico typically associated with negative effects in Florida and Texas coastal waters (Tester and Steidinger 1997; Brown et al. 2006; Magaña and Villareal 2006). Louisiana normally does not experience *K. brevis* blooms due to its low-salinity waters; however, both in 1996 and 2015, *K. brevis* blooms developed east of the Mississippi River, resulting in the closure of oyster beds (Dr. Q. Dortch personal communication for 1996; Bargu unpublished data for 2015).

Other toxic HAB species, *P. minimum* and *H. akashiwo*, were also detected in very high abundance shortly after Deepwater Horizon oil spill (Bargu unpublished data). Despite the potential for a reduction in diatoms on the Louisiana shelf because of the decline in the Si:N atomic ratio from 4:1 over several decades (Turner and Rabalais 1991; Dortch et al. 2001), the community response has been a shift from more heavily silicified diatoms to less silicified diatoms including the toxic *Pseudo-nitzschia* species (Rabalais et al. 1996; Dortch et al. 2001). In fact, the flux of diatoms (Turner and Rabalais 1994) and the abundance of *Pseudo-nitzschia* (Parsons et al. 2002) are reported to increase along with the increasing concentration of nitrate-N and the load of nitrate-N in the river, while the Si:N ratio decreased.

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The diatom *Pseudo-nitzschia* is a common coastal and oceanic phytoplankton with worldwide distribution (Hasle 2002; Liefer et al. 2013). Multiple species in the genus are capable of producing domoic acid (DA), which is a neurotoxic amino acid responsible for deaths of higher trophic-level organisms, including humans (Wright et al. 1989; Bates 2000; Scholin et al. 2000; Bates and Trainer 2006). Several lines of evidence indicate that *Pseudo-nitzschia* growth and toxicity are stimulated by high-nutrient inputs either from peaked river discharge (Dortch et al. 1997; Parsons et al. 2002; Liefer et al. 2009) or from upwelling events (Trainer et al. 2000; Kudela et al. 2005), thereby increasing the potential for DA-poisoning incidents where nutrient enrichment have occurred. On the other hand, several other field studies have demonstrated no strong correlations or negative correlations between *Pseudo-nitzschia* cell abundance, its toxicity, and dissolved inorganic nutrients and/or their elemental ratios (Schnetzer et al. 2007; Trainer et al. 2009a, b).

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Pseudo-nitzschia spp. in Louisiana coastal waters tend to bloom in the early spring ($>10^6$ cells l^{-1}) (Dortch et al. 1997; Parsons et al. 1999; Parsons et al. 2013) before the macro-zooplankton population is well established (Dagg 1995). Cells of *Pseudo-nitzschia* that are not grazed in the upper water column are likely to contribute to the carbon flux to the bottom water and seafloor, as suggested by Dortch et al. (1997) and verified by Baustian et al. (2011). The intact cells that are capable of producing the toxin DA could also influence the sediment toxin pool and eventually be consumed by organisms like polychaetes (Baustian et al. unpublished data). DA production has been documented in two *P.* cf. *pseudodelicatissima* clones isolated from local waters (Parsons et al. 1999; Pan et al. 2001), and *P. calliantha*, *P. delicatissima*, *P. pseudodelicatissima*, and *P. multiseries* were suggested as DA producers based on their dominance in the natural samples analyzed (Parsons et al. 1999; Del Rio et al. 2010; Parsons et al. 2013). Plankton-feeding organisms in the region, such as oysters and menhaden, are known vectors of DA to higher trophic levels, including humans (Thessen et al. 2005; Del Rio et al. 2010).

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Reducing the frequency of *Pseudo-nitzschia* occurrences and minimizing their environmental impacts will require an understanding of the environmental conditions that regulate their distribution, abundance, and toxicity. However, the ecology of these diatoms and the consequences of DA in the food web of the northern Gulf of Mexico remain poorly understood including, specifically, what controls their abundance and toxicity. This study focuses on the abundance and toxicity of the diatom *Pseudo-nitzschia* in surface waters on a monthly basis over 3 years along a transect of stations located west of the Mississippi River Delta. Louisiana estuaries and coastal waters provide habitats for many filter-feeding, phytoplankton-consuming organisms. Understanding the variability in the environmental parameters affecting DA production is critical for the future management of these habitats and protecting vulnerable marine vertebrates, including endangered species of seabirds and mammals that can forage on organisms potentially contaminated with DA, and can support specific management of land use practices in the Mississippi River watershed.

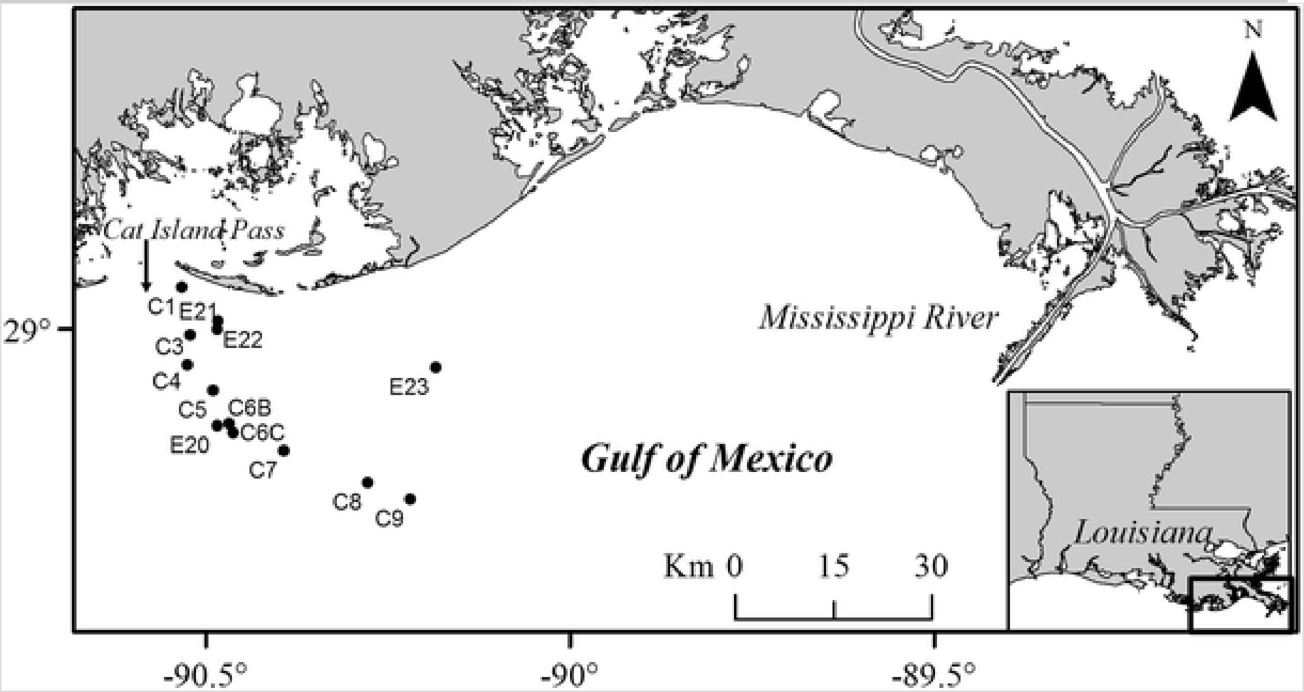
Material and Methods

Collection of Oceanographic Data and Water Sampling

Surface water samples were collected from a transect off Cat Island Pass, LA, that had nine stations (the depths along transect stations ranged from 5 to 30 m) in 2007 monthly except February, in 2008 monthly, and in 2009 monthly except November and December (Fig. 1). Surface water samples from four additional stations (E20 to E23) near or along the same transect were collected in April of 2007 and 2008 only (Fig. 1). Each station included a phytoplankton net tow (20-µm Nitex mesh), a SeaBird CTD cast, and a surface water sample for measuring the cell abundance of *Pseudo-nitzschia*, levels of particulate (within the cell) domoic acid (P-DA), chlorophyll a (Chl *a*), and dissolved inorganic nutrients. Additional data on temperature and salinity were collected with a YSI 6820 near the surface. Mississippi River discharge data were collected by the US Army Corp of Engineers (http://www2.mvn.usace.army.mil/cgi-bin/wcmanual.pl?01100) at Tarbert Landing, MS.

Fig. 1

Map of the study area in the northern Gulf of Mexico showing the main sampling "C" transect located south of Cat Island Pass and extra stations labeled as "E." C transect stations were sampled for all 3 years (2007, 2008, and 2009), while "E" stations were only sampled in April 2007 and 2008



Chlorophyll *a* and Nutrient Analyses

The concentrations of Chl *a* were determined at all stations as an estimate of phytoplankton biomass. Seawater (25 to 100 ml) was filtered onto 25-mm GF/F filters (Whatman) and extracted in the dark for a minimum of 2 h in

40/60 DMSO/90 % aqueous acetone before analysis on a Turner fluorometer (Parsons et al. 1984). Samples for nutrient analysis were frozen on board for later analysis in the laboratory. Water samples for dissolved inorganic nutrients were also analyzed at all stations. Unfiltered samples were analyzed for DIN (ammonium, nitrate, and nitrite), orthophosphate (DIP), and silicate using EPA methodology (353.2, 350.1, and 365.2; USEPA 1993) using a Lachat auto-analyzer II system (8000 series) equipped with an autosampler (ASX-400 series). The data are reported for nitrate + nitrite concentration, and the nitrite concentration was <1.0 μM, which compares to an order of magnitude higher nitrate concentration. The nitrate + nitrite concentration is, therefore, almost entirely nitrate. The data and metadata are archived at http://data.nodc.noaa.gov/accession/0060060 for 2007, http://data.nodc.noaa.gov/accession/0069471 for 2008, and http://data.nodc.noaa.gov/accession/0099531 for 2009.

Pseudo-nitzschia Enumeration and Species Identification in Water Samples

Water and net tow samples were stored on ice and processed immediately upon delivery to the laboratory, which was normally within 2–3 h. A portion of the water sample was preserved in 2 % gluteraldehyde and stored at 4 °C until analyzed for *Pseudo-nitzschia* cell enumeration. The total number of *Pseudo-nitzschia* cells from water samples were counted in replicates using a gridded Sedgewick-Rafter 1-mm^2 counting chamber and a Zeiss Axio Observer-A1 inverted microscope with epifluorescence capability. *Pseudo-nitzschia* spp. cells were counted until ~200 cells were reached, and then, the number of counted grids was recorded and used in a calculation to determine cells ml^{-1}. For cell counts between 10 and 200 cells, the entire counting chamber was searched. If less than 10 cells were counted, 5–20 ml of the sample was stained with proflavin (Sigma-Aldrich), filtered, and recounted using epifluorescence microscopy on the Zeiss inverted microscope.

Additional morphological observations were made by electron microscopy for the taxonomic diversity of *Pseudo-nitzschia* spp. for selected samples from each month over the 3 years. To ensure accurate identification of *Pseudo-nitzschia* species, the frustules were cleaned with strong oxidizing agents ($KMnO_4$ and concentrated HCl) using standard electron microscopy sample preparation methods (Bargu et al. 2002a, b). The filters were mounted onto aluminum stubs with double-sided tape for scanning electron microscopy (SEM) observations, and mounted filters were air-dried in a desiccator for 24 h and sputter coated with gold palladium. All SEM micrographs were obtained with a Cambridge Stereoscan 260 scanning electron microscope at 10 kV. For transmission electron microscopy (TEM) analysis of the frustules, a drop of cleaned material was affixed onto a 100-mesh copper grid with a formvar carbon support film. Finally, the samples were placed in a desiccator to dry before viewing them on a Jeol 100CX TEM.

Domoic Acid Detection with cELISA Method

Additional portions of the water were vacuum-filtered through 25-mm diameter and 0.7-μm GF/F filters (Whatman) for P-DA measurements. Up to 500 ml of water (two replicates) were filtered for each replicate of P-DA and then stored in a 2-ml microcentrifuge tube at −20 °C until analysis. The filters were extracted with 10 ml of 20 % methanol (MeOH) in 15-ml centrifuge tubes, sonicated (2 min, 30–40 W) in an ice bath with a Sonicator 3000 equipped with a microtip (Misonix), and then centrifuged (10 min, 1399×*g*). The supernatant was collected and passed through a 0.22-μm syringe filter (Corning) into a clean 15-ml centrifuge tube. Samples were temporarily stored at −20 °C until analysis. A spike and recovery experiment was performed on water samples before the analysis. The spiked and control samples were extracted using the same procedure as above, and the percent recoveries were calculated. A competitive enzyme-linked immunosorbent assay (ASP Direct cELISA Kit, Biosense Laboratories AS, Norway) was used to quantify DA concentrations. Each sample was run in duplicate at multiple dilutions according to the manufacturer’s specifications. The absorbance of the sample was read at 450-nm wavelength. Cellular DA (C-DA) levels were calculated from P-DA levels divided by total *Pseudo-nitzschia* cell numbers. Note that the C-DA levels were calculated based on the assumption of all cells being equally toxic.

Statistical Analysis

The abundance of *Pseudo-nitzschia*; the concentrations of P-DA, Chl *a*, ammonium, nitrate + nitrite, silicate, orthophosphate, DIN:DIP, Si:DIN, and Si:DIP; and the MSR discharge data were log-transformed to meet assumptions of normality and homogeneity to test for statistical significance. Transformations for normality were not used for the temperature and salinity data. Tests were conducted with an alpha = 0.05 in SAS 9.4 (SAS Institute Inc. 2013). The relationships among the data were investigated using simple linear regressions and Pearson's correlations with coefficients between −1 and 1, signifying perfect negative or positive correlation. Data sets with periods of high MSR discharge (1 January to 30 June) and low MSR discharge (1 July to 31 December) were created to determine if the MSR discharge influenced the environmental conditions of *Pseudo-nitzschia* communities. *T* tests with Bonferroni adjustment for multiple tests were used to determine if periods of high MSR flow differed from periods of low MSR flow, and an ANOVA with post hoc Tukey-Kramer test was run for pairwise comparisons of high-river seasons (2007, 2008, and 2009).

Results

Monthly surface water samples were taken from multiple stations along a transect in Louisiana coastal waters over a 3-year period. *Pseudo-nitzschia* was frequently present (92 %, $n = 194$) throughout the year and had cell abundances typically exceeding 10^4 cells l^{-1} (Fig. 2). Cell concentrations close to or greater than 10^6 cells l^{-1} were indicative of blooms, which occurred mainly in early spring months in all 3 years (Fig. 3a). P-DA was also detected regularly (77 %, $n = 217$; Fig. 3b), and the concentrations were highly correlated with cell abundances of *Pseudo-nitzschia* ($r = 0.65$, $p < 0.001$, $n = 182$), although some blooms (i.e., spring 2007 and 2009) had higher toxin levels than others (i.e., spring 2008; see Fig. 3a, b and Table 2). The maximum P-DA concentration was found to be 13.4 µg DA l^{-1} in April 2007, when the cell numbers were higher than 10^6 cells l^{-1}. The Chl *a* levels varied from 0.2 to 44 µg l^{-1} over the 3 years, with the highest concentrations detected in April and August of 2008 (Fig. 3d). The Chl *a* levels were positively correlated with MSR discharge ($r = 0.33$, $p < 0.001$, $n = 219$), and *Pseudo-nitzschia* abundance did not account for much of the variation in Chl *a* levels for the combined 3 years ($r^2 = 0.04$, $p = 0.01$, $n = 187$). Based on the electron microscopy observations, the dominant *Pseudo-nitzschia* species were the *P. pseudodelicatissima* complex, consisting mainly of *P. calliantha* and P. *pseudodelicatissima* for all 3 years (Fig. 4). Both species have been previously reported as toxin producers in Louisiana coastal waters (Del Rio et al. 2010; Parsons et al. 2013). *P. delicatissima*, *P. multiseries*, and *P. pungens* were also observed but at lower presence.

Fig. 2

Time series data of *Pseudo-nitzschia* cell abundance (log scale, cells l^{-1}) from 2007 to 2009, for all stations ($n = 194$)

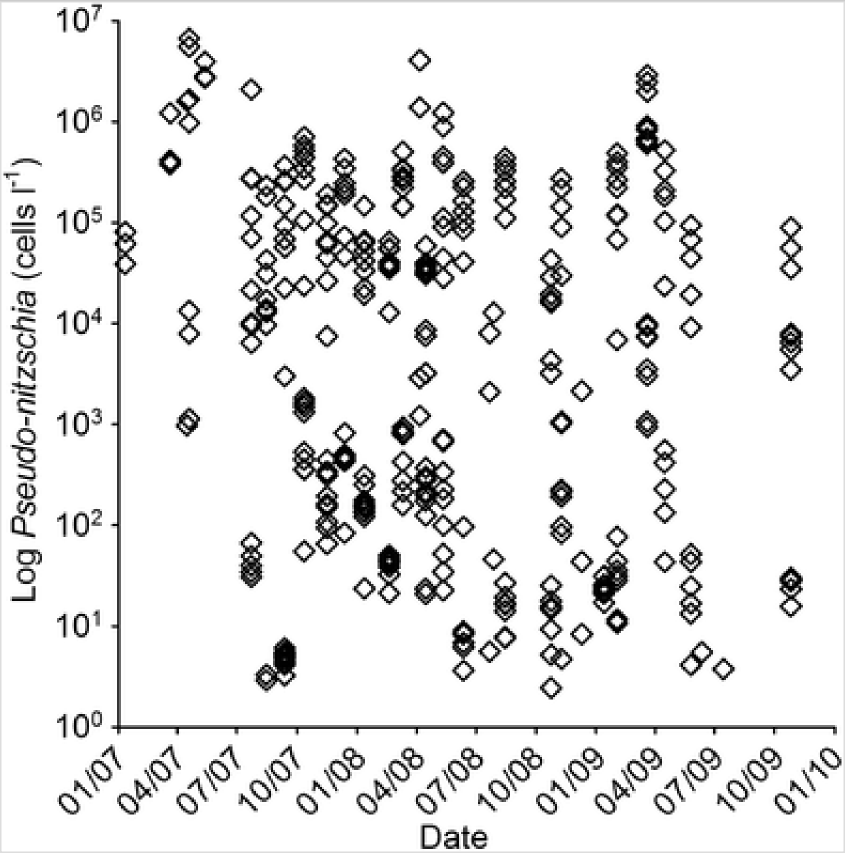


Fig. 3

Time series data, from 2007 to 2009, for all stations: **a** abundance of *Pseudo-nitzschia* cells (cells l^{-1}), **b** particulate domoic acid concentrations (P-DA; µg l^{-1}), **c** cellular domoic acid levels (C-DA; pg cell l^{-1}), and phytoplankton biomass (Chl *a*; µg l^{-1}). All *y* axes are on a linear scale

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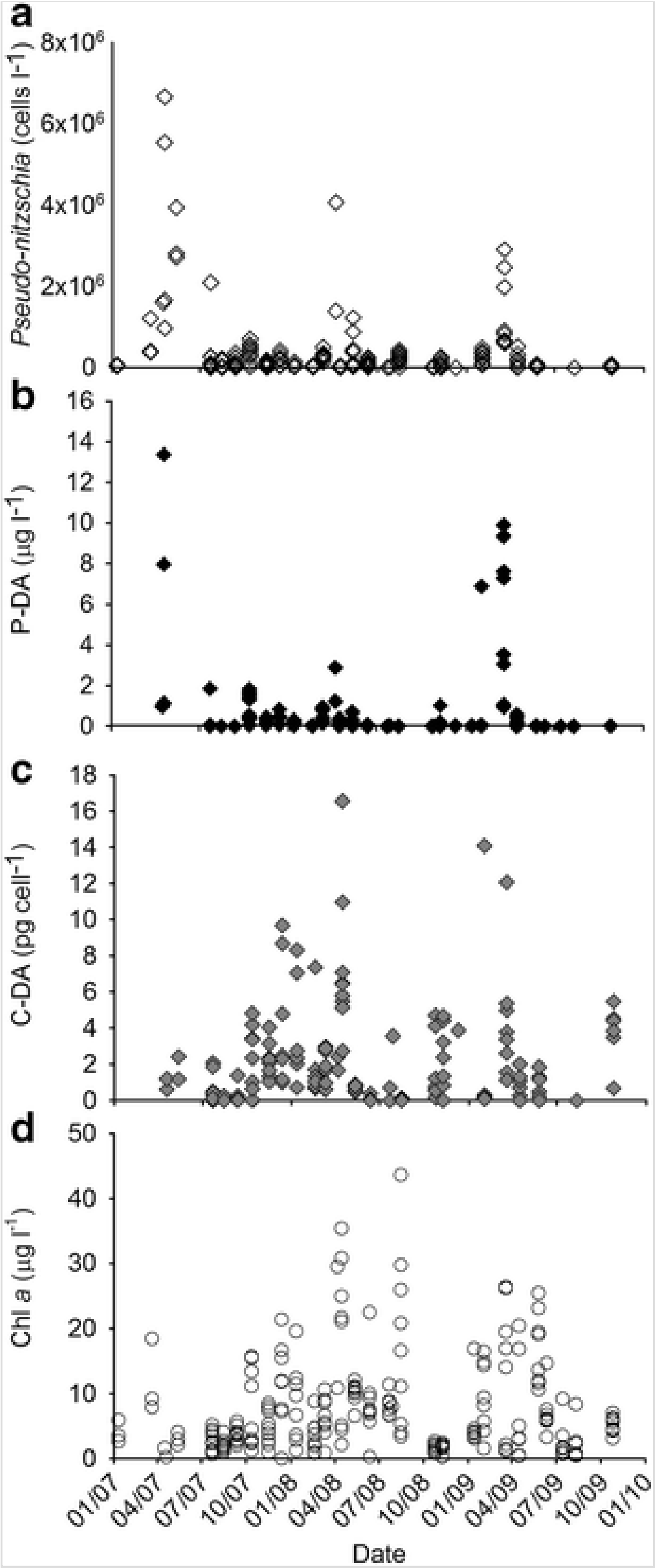
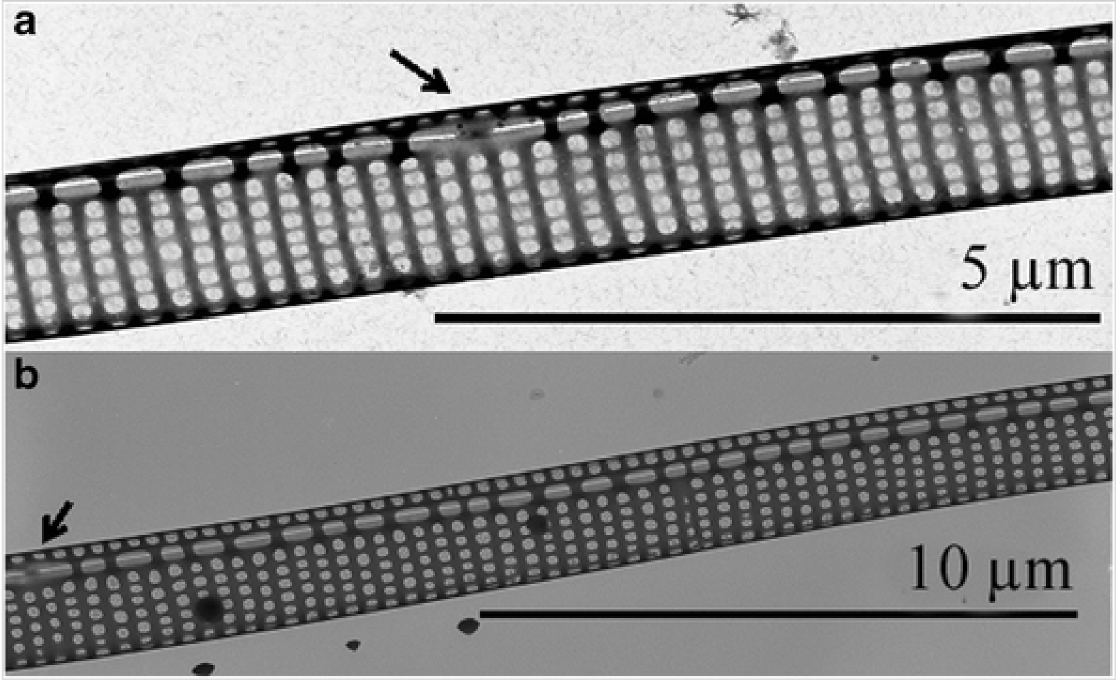


Fig. 4

Transmission electron microscopy images of **a** *Pseudo-nitzschia pseudodelicatissima* and **b** *P. calliantha* from the surface water in May 2007. These were the two most abundant *Pseudo-nitzschia* species found in Louisiana coastal waters during the 3 years. Notice the presence of single row of poroids within the striae, with differences in sectors of poroid hymen for the two species. A central nodulus is present in both (*see arrows*)



The concentrations of C-DA were calculated from total *Pseudo-nitzschia* abundance, assuming that all cells are equally toxic. Even though in all 3 years, the dominant *Pseudo-nitzschia* species were the *P. pseudodelicatissima* complex, the C-DA data still needs to be evaluated cautiously. Overall, C-DA levels were highly variable and ranged from 0.01 to 16.6 pg DA cell^{-1} (Fig. 3c). The highest concentrations were detected in April 2008 when the associated *Pseudo-nitzschia* cell numbers were relatively low (~3 × 10^{5} cells l^{-1}; Fig. 3a) and were high in spring 2009 (Fig. 3c). The average C-DA concentration was only 1.3 ± 0.8 in spring 2007, when the *Pseudo-nitzschia* cells were the most abundant (Fig. 3a, c) and when the corresponding P-DA concentration was high (Fig. 3b).

The environmental conditions were highly variable among the 3 years, and especially between high MSR flow (January 1 to June 30) and low MSR flow (July 1 to December 31) periods, likely due to the timing and the volume of the MSR discharge that is driven by continental weather patterns, precipitation, and land use. In general, over the 3 years, the high MSR flow was characterized by cooler temperatures, lower salinity, higher dissolved inorganic nutrient concentrations (especially nitrate + nitrite), lower Si:DIN, higher DIN:DIP, and higher MSR discharge compared to the low MSR flow period (Table 1). Correspondingly, phytoplankton biomass (Chl *a*), *Pseudo-nitzschia* abundance, and P-DA concentrations were significantly higher in the high MSR flow period compared to the low MSR flow period (*t* tests, $p < 0.003$; Table 1).

Table 1

Summary statistics of surface water environmental parameters, collected from C + E (January 1 to June 30) compared to low MSR flow (July 1 to December 31) for the comb Gulf of Mexico

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	High MSR flow					
	Mean	**Standard error**	**Minimum**	**Maximum**	**Number**	**Mean**
Temp (YSI) (°C)[a]	21.9	0.4	15.8	31.4	116	27.3
Salinity (YSI) (ppt)	27.3	0.4	18.2	35.5	116	28.8
Chl *a* (µg 1^{-1})[a]	9.3	0.7	0.2	35.4	109	5.5
Ammonium (µM)	1.5	0.3	0.3	30.2	115	1.2
Silicate (µM)	10.6	0.8	0.6	38.8	113	10.3
Nitrate + nitrite (µM)[a]	8.3	0.6	0.0	19.4	115	2.2
Orthophosphate (µM)	0.8	0.0	0.3	3.9	113	0.7
DIN:DIP[a]	15.2	1.3	0.9	50.5	113	5.1
Si:DIN[a]	1.6	0.2	0.1	21.6	113	7.1
Si:DIP	18.3	2.0	0.8	98.0	113	17.9
MSR discharge ($m^3 s^{-1}$ × 1000)[a]	22.7	0.8	10.3	39.6	116	11.1
Pseudo nitzchia (cells 1^{-1})[a]	5.6×10^5	1.1×10^5	0.0	6.7×10^5	100	1.5×10^5
P-DA (µg 1^{-1})[a]	0.8	0.2	0.0	13.4	106	0.1

[a] Significant difference between the parameters in high and low MSR flow periods (*t*

However, differences were noticed among the years' high MSR flow period due to changes in the timing, duration, and river discharge and delivered dissolved inorganic nutrient concentrations (Table 2). The coastal waters during the high MSR flow period in 2007 received the lowest mean MSR discharge flow, and correspondingly, it was most saline with the lowest Chl *a* levels observed. The coastal waters in 2008, during high MSR flow period, received the highest MSR discharge flow, and correspondingly were of lower salinity, and had the highest concentrations of nitrate + nitrite, ammonium, silica, and DIN:DIP and higher Chl *a*. Similar to 2007, MSR discharge in 2009 during the high MSR flow period was initially low during the month of March (mean = $16.2 \times 1000\ \mathrm{m}^3\ \mathrm{s}^{-1}$, $n = 31$), started to increase in April (mean = $21.7 \times 1000\ \mathrm{m}^3\ \mathrm{s}^{-1}$, $n = 30$), and increased significantly in May (mean = $29.4 \times 1000\ \mathrm{m}^3\ \mathrm{s}^{-1}$, $n = 31$). The highest orthophosphate and lowest nitrate + nitrite concentrations and DIN:DIP ratios were observed in the sampled waters during the high MSR flow period in 2009, compared to 2007 and 2008, but similar Chl *a* levels were detected in 2008 and 2009 (Table 2).

Table 2

Summary statistics of surface water environmental parameters collected from C + E st
stations during high Mississippi River (MSR) flow (January 1 to June 30) of 2009 in the

	High MSR flow—2007					
	Mean	**Standard error**	**Minimum**	**Maximum**	**Number**	**Mean**
Temp (YSI) (°C)	21.4	0.9	17.4	27.6	14	21.9
Salinity (YSI) (ppt)	29.4	0.4	26.9	31.7	14	27.0
Chl *a* (μg 1^{-1})	5.3	1.5	0.2	18.4	11	9.6
Ammonium (μM)	1.0	0.3	0.3	5.1	14	2.0
Silicate (μM)[a]	7.2^{A}	1.6	1.2	18.7	14	14.3^{B}

Nitrate + nitrite (µM)	9.1	1.5	2.2	19.2	14	10.0
Orthophosphate (µM)^{a}	0.7^{AB}	0.0	0.5	0.9	14	0.6^{A}
DIN:DIP^{a}	14.5^{AB}	2.3	4.0	33.9	14	22^{A}
Si:DIN	0.8	0.2	0.1	2.1	14	1.7
Si:DIP^{a}	9.8	2.0	1.6	23.6	14	29.5
MSR discharge (m^3 s^{-1} × 1000)	17.6	0.3	16.3	19.4	14	24.8
Pseudo-nitzschia (cells 1^{-1})^{a}	2.0×10^{6A}	5.6×10^5	3.9×10^5	6.7×10^6	14	2.5×10^{5B}
P-DA (µ 1^{-1})^{a}	5.9^{A}	3.0	1.0	13.4	4	0.3^{B}

A, B, and AB share of superscript letters indicate no significant difference between th

[a] Significant difference among one of the years of high MSR flow periods (ANOVA)

The highest *Pseudo-nitzschia* cell abundances were observed mainly in 2007 during the high MSR flow period (but at the lowest discharge among the 3 years) and in earlier (late February and March) months of 2009 when MSR discharge flow was mid-range (<20,000 m^3 s^{-1}; Fig. 5a, c). The abundance of *Pseudo-nitzschia* cells was negatively correlated with MSR discharge ($r = -0.28$, $p = 0.005$, $n = 100$; Table 3). Consequently, during the high MSR flow period, the coastal waters contained the highest P-DA levels, which were correlated negatively with temperature ($r = -0.43$, $p = 0.0001$, $n = 106$) and Si:DIN ($r = -0.29$, $p = 0.003$, $n = 103$) and positively with salinity ($r = 0.28$, $p = 0.004$, $n = 106$), silicate ($r = 0.20$, $p = 0.04$, $n = 103$), and nitrate + nitrite ($r = 0.34$, $p = 0.0004$, $n = 105$) concentrations and DIN:DIP ($r = 0.34$, $p = 0.0004$, $n = 103$; Table 3).

Fig. 5

Pseudo-nitzschia abundances (cells l^{-1}) and particulate domoic acid (P-DA; µg l^{-1}) concentrations in the northern Gulf of Mexico during high Mississippi River (MSR) flow period in **a** 2007, **b** 2008, and **c** 2009 in relation to MSR discharge

AQ7

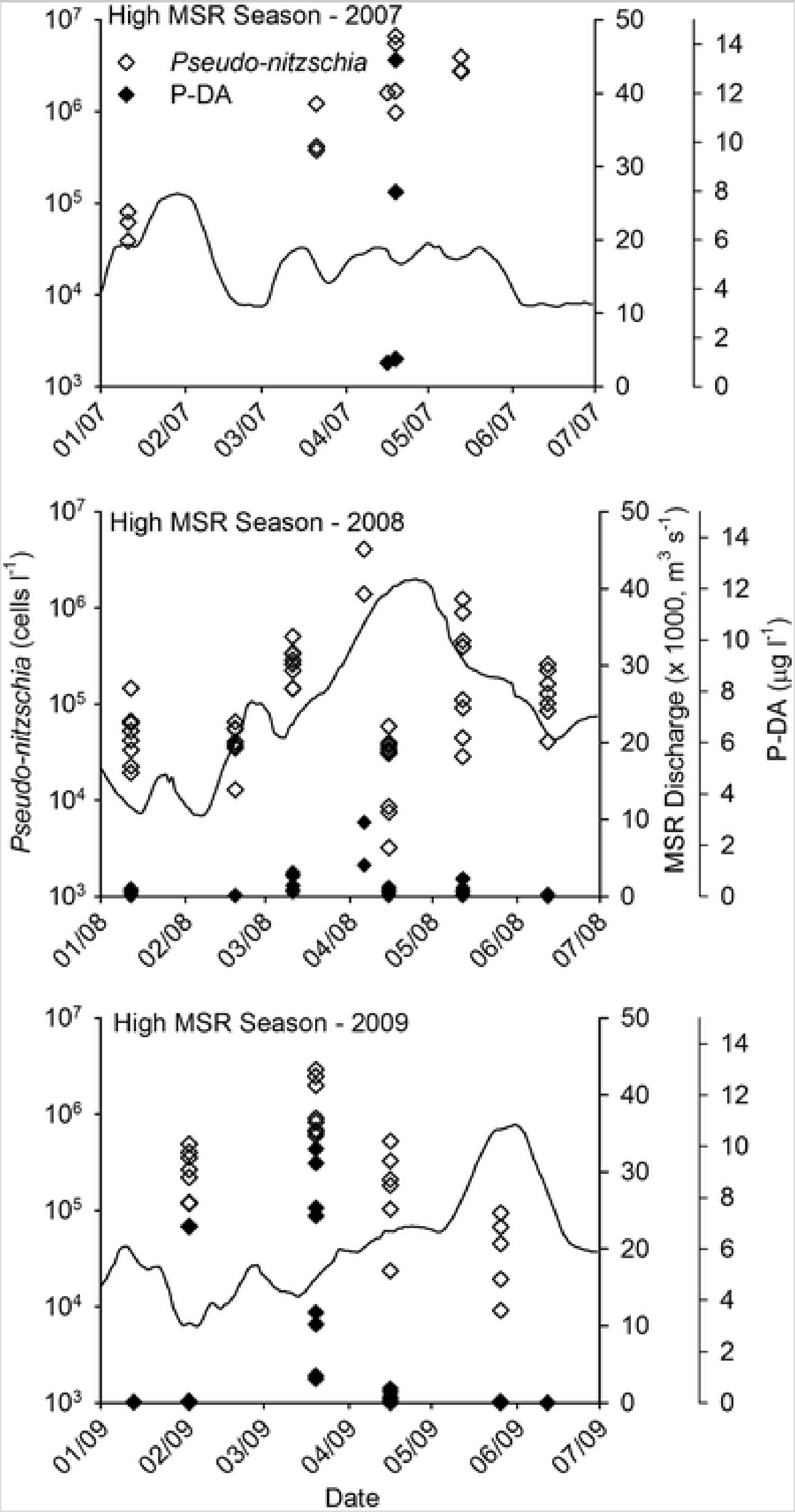

Table 3

Pearson’s correlation coefficients of surface water environmental parameters and biotic 30) for the combined years of 2007, 2008, and 2009 in the northern Gulf of Mexico

	Temperature	**Salinity**	***Pseudo-nitzschia***	**P-DA**	**Chl *a***	**Ammoniu**
Temperature						
Salinity	−0.48*					
Pseudo-nitzschia	−0.09	0.12				
P-DA	−0.43*	0.28*	0.70*			
Chl *a*	0.11	−0.52*	0.17	0.18		
Ammonium	−0.03	0.039	0.12	0.16	0.14	
Silicate	−0.18*	−0.37*	−0.16	0.20*	0.43*	0.10
Nitrate + nitrite	−0.46*	−0.19	−0.01	0.34	0.11	−0.05
Orthophosphate	−0.11	0.25*	0.03	0.01	−0.06	0.07
DIN:DIP	−0.37*	−0.28*	0.01	0.34*	0.17	0.24*
Si:DIN	0.43*	−0.24*	−0.19	−0.29*	0.27*	−0.24*
Sir:DIP	−0.12	−0.41	−0.15	0.17	0.40*	0.06
MSR	0.48*	−0.52*	−0.28*	−0.18	0.25*	0.14

*Significant difference ($p < 0.05$)

Discussion

The continental shelf of the northern Gulf of Mexico is physically and biologically dominated by the Mississippi River. It is one of the world's largest rivers in terms of freshwater discharge, and water flowing from it is turbid and rich in nutrients; the concentrations of nitrate and silicate at the river mouth can exceed 100 µM (Turner and Rabalais 1991). Similar to our study, previous studies reported *Pseudo-nitzschia* bloom occurrences in Louisiana coastal waters during the spring months when the MSR discharge was comparatively high (Dortch et al. 1997; Parsons et al. 2013); however, differences in the timing of bloom occurrences and their correlations to nutrient input varied in these studies and in ours. A multiyear study by Dortch et al. (1997) have shown high cell abundances of *Pseudo-nitzschia* both at the surface layer and bottom waters corresponding to the average maximum in river flow; however, they also observed highest cell numbers associated with the lowest-nutrient concentrations. On the other hand, a longer-term study (13 years) by Parsons et al. (2013) detected highest abundances of *Pseudo-nitzschia* in Louisiana coastal waters when DIN concentrations were high and both silicate and DIP concentrations were low in early spring months. Similar to Parsons et al. (2013), Liefer et al. (2009) observed highest cell abundances after the peaks in river discharge with high DIN levels in Alabama coastal waters in the northern Gulf of Mexico.

In our study, differences in *Pseudo-nitzschia* peak times and its toxicity were correlated mainly with the timing and magnitude of MSR discharge and changes in associated parameters such as nutrient stoichiometry and salinity. A negative relationship between high MSR discharge and *Pseudo-nitzschia* blooms was documented. *Pseudo-nitzschia* became most abundant when the surface waters started to warm in early spring, but the MSR discharge was not at its highest, and consequently, salinity was high and dissolved inorganic nutrient concentrations were comparatively low. Further, *Pseudo-nitzschia* did not correlate with Chl *a* concentrations and high Chl *a* levels were positively correlated with MSR discharge (Table 3). An unusual increase in several potentially toxin-producing dinoflagellate species dominated by *Dinophysis* spp. and *Prorocentrum* spp. was observed in 2008 during the high MSR flow period (data are not shown) when the *Pseudo-nitzschia* abundance was relatively low (Table 2). High-river discharges were also previously associated with phytoplankton communities other than *Pseudo-nitzschia* in Southern California waters (Schnetzer et al. 2007; Schnetzer et al. 2013).

In other field studies, high *Pseudo-nitzschia* cell abundances were also found to be associated with low-nutrient conditions. The *Pseudo-nitzschia* density was found to be highest in the most saline, clear, and nutrient-poor waters of Alabama with strong negative correlation between *Pseudo-nitzschia* and Si:DIN (Liefer et al. 2013). Schnetzer et al. (2007) also found negative correlations between P-DA; *Pseudo-nitzschia*; and the concentrations of Si, DIN, and DIP in the Southern California waters and the ratios of Si:DIP and DIN:DIP. Contradictorily, Trainer et al. (2009a) have shown no correlation between *Pseudo-nitzschia*, P-DA, and dissolved inorganic nutrients in coastal waters of Pacific Northwest. As suggested by Schnetzer et al. (2007) as well, low-nutrient conditions when *Pseudo-nitzschia* abundance and particulate DA concentrations were high in our study might also be due to lag effects of *Pseudo-nitzschia* already having drawn down the nutrients, and the sampling occurred in the aftermath of the conditions that triggered growth. However, looking at Fig. 5, there is one main peak of river discharge per period and *Pseudo-nitzschia* peaks do not correspond to peak riverine discharge periods.

There was a strong correlation between *Pseudo-nitzschia* and P-DA concentrations in our study; however, C-DA levels were highly variable. The toxin content of a *Pseudo-nitzschia* cell responds differently than population abundances as the cell is dependent on strain, species, and growth phase but also on environmental conditions and stress level. In all 3 years, spring *Pseudo-nitzschia* species communities were similar. The dominant *Pseudo-nitzschia* species were the *P. pseudodelicatissima* complex, consisting mainly of *P. calliantha* and *P. pseudodelicatissima*. Potentially toxic species of *Pseudo-nitzschia*, including *P. pseudodelicatissima*, *P. calliantha*, *P. multiseries*, and *P. delicatissima*, have been identified in the northern Gulf of Mexico (Fryxell et al. 1990; Parsons et al. 1998; Parsons et al. 1999; Pan et al. 2001; Del Rio et al. 2010; Parsons et al. 2013). *P. pseudodelicatissima* and *P. calliantha* are generally the dominant species reported in these studies and our current study, and *P*. cf. *pseudodelicatissima* was found throughout the entire salinity range in the region (Thessen et al. 2005). Both species have been reported as toxin producers in Louisiana coastal waters (Del Rio et al. 2010; Parsons et al. 2013), but species-specific toxin production is unknown. Laboratory experiments have identified nutrient stress, especially phosphorus, as one of the primary triggers of the DA cellular production by the genus *Pseudo-nitzschia* (Bates et al. 1991; Pan et al. 1996a, b; Fehling et al. 2004). Similarly, field observations have suggested phosphorus stress in DA production due to negative correlation found between C-DA and DIN:DIP and Si:DIP ratios (Schnetzer et al. 2007; Trainer et al. 2009a, b).

Phosphorus limitation, as generally defined by high DIN:DIP due to excess concentrations of nitrate entering coastal waters from MSR discharge, was noticeable during high MSR flow period among the 3 years sampled. Highest limitation likely occurred in spring 2008, the year with highest MSR discharge observed, with in situ DIN:DIP ratio of 22 and Si:DIP ratio of 29.5

corresponding to highest C-DA detections. However, overall P-DA concentrations in the water were low due to low *Pseudo-nitzschia* cell abundances, even though the individual cells themselves were potentially more toxic.

High *Pseudo-nitzschia* abundances and P-DA concentrations can be seen not only in earlier months of spring when MSR discharge is rising but also could be observed in drought years when the MSR discharge is comparatively low. The upper part of the Mississippi River watershed, where most of the current N and P loads originate (Alexander et al. 2008), falls within the area of North America predicted to experience higher runoff (higher precipitation) and earlier peak discharge (earlier snow melt in a warming climate) (Rabalais et al. 2010; Rabalais et al. 2014). This increased precipitation should result in increased erosion, the loss of phosphorus, and the increased flux of dissolved inorganic nitrogen, particularly nitrate, through the soils and artificially drained agriculture areas. The combination of increased nutrient loads (anticipated rise in human activities) and increased freshwater discharge (from IPCC-estimated increase in precipitation and earlier snow melt in the upper Mississippi River basin) (Rabalais et al. 2009; Rabalais et al. 2010) will aggravate the already high loads of nitrogen from the Mississippi River to the northern Gulf of Mexico. The abundance of primary production, of which *Pseudo-nitzschia* is a major contributor among others (Dortch et al. 2001; Parsons et al. 2007), responds to higher nutrient loads (Lohrenz et al. 1997; Rabalais et al. 2002), which will increase the organic matter flux to the lower water column. The strengthened water column stratification (Wiseman et al. 1997), i.e., without increasing strength and frequency of tropical storms, will reduce the dissolved oxygen diffusing from the upper water column to the lower water column and will aggravate hypoxia.

AQ8

Even though the P-DA concentrations in this study are the same magnitude as those recorded during California *Pseudo-nitzschia* blooms associated with animal mortalities (Scholin et al. 2000; Bargu et al. 2002a; Lefebvre et al. 2002), there have been no reported mass mortalities of birds or marine mammals in Louisiana waters due to acute DA toxicity. On the other hand, DA has been detected in gulf menhaden (*Brevoortia patronus*) from Terrebonne Bay, LA, a planktivorous fish that is the target of the second largest fishery in the USA by tonnage (Del Rio et al. 2010), and in multiple other recreationally and commercially important fish species collected from Alabama coastal waters (Liefer et al. 2013).

The puzzling lack of massive pelagic animal strandings in the waters of the Louisiana coast may be partially explained by the temporally displaced grazing by macro-zooplankton (Dagg 1995) that influences particulate flux to bottom. Early spring months when coastal water temperatures are still cold and river discharge starts to peak, *Pseudo-nitzschia* blooms can occur as seen in 2007, before copepods can be abundant enough to reduce their abundance via grazing. When the potential mismatch happens, *Pseudo-nitzschia* and their toxin could sink directly or flocculate at the end of their blooms and sink more rapidly (Dortch et al. 1997; Sekula-Wood et al. 2009; Silver et al. 2010). On average, 46 % of the *Pseudo-nitzschia* present in a surface water layer of coastal Louisiana was found to sink into the trap each day (Dortch et al. 1997; Dortch et al. 2001). The rapid settlement of *Pseudo-nitzschia* cells onto the shallow shelf can be a source of DA for benthic organisms. High concentrations of DA (as much as 700 μg DA per g tissue) have been documented in near-shore and intertidal filter- and deposit-feeding benthic communities (Kvitek et al. 2008; Vale and Sampayo 2001). Baustian et al. (unpublished data) found that *Pseudo-nitzschia* spp. made up more than 15 % of the gut contents for a common surface-deposit feeding polychaete (at 20-m water depth) during spring collections in 2003 and 2004 from the same C transect that was sampled in this study. Little attention has been afforded to the flux of toxin-producing phytoplankton and the incorporation of their toxins into the benthic food web with potential for trophic transfer. Future studies in shallow waters adjacent to the Mississippi River are needed to better understand the contribution of *Pseudo-nitzschia* and associated DA concentrations to the sediments.

Understanding the factors important in toxic *Pseudo-nitzschia* bloom dynamics is important to the management of coastal fishery resources and for the safety of a significant portion of the US seafood production associated with the Mississippi River Delta. The data we present in this paper document higher DA concentrations than previously measured, a more temporally complete data series, and implied relationships with MSR fluxes of freshwater and its associated constituents. Our results and others in the future can facilitate and inform coastal and watershed management actions that mitigate nutrient loads to the Gulf of Mexico.

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