

1 The polychaete, *Paraprionospio pinnata*, is a likely vector of domoic acid to the benthic food  
2 web in the northern Gulf of Mexico

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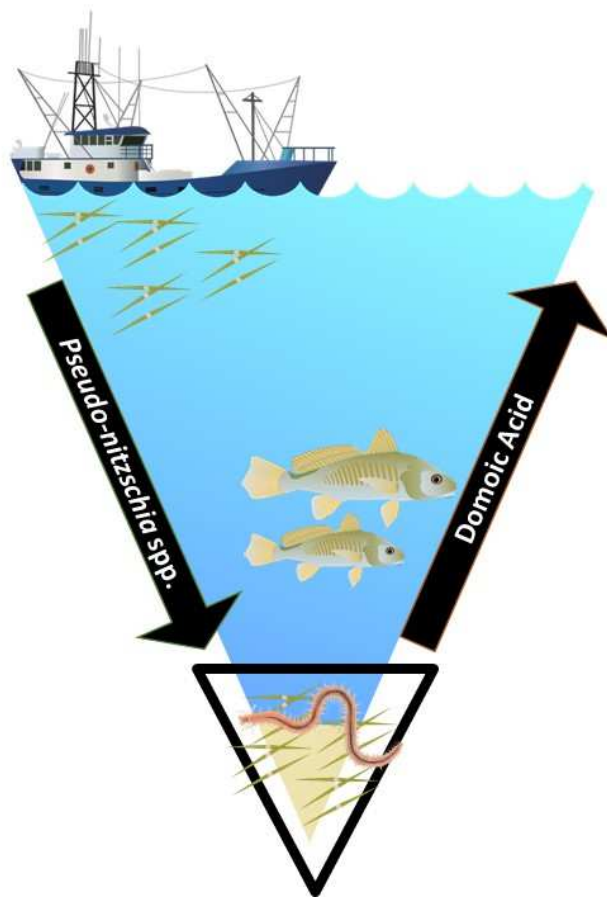
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27 **Graphical Abstract**



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35 **Highlights**

- 36 • *Pseudo-nitzschia* spp. are found in surface waters, bottom waters, and sediments
- 37 • Known toxic species of *Pseudo-nitzschia* are in the surface sediments
- 38 • Gut contents of the polychaete, *Paraprionospio pinnata*, indicate ingestion of *Pseudo-*
- 39 *nitzschia* spp.
- 40 • Trophic transfer of toxic *Pseudo-nitzschia* spp. into benthic food webs is likely

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53 **Abstract**

54 A somewhat disparate, yet temporally cohesive, set of phytoplankton abundance,  
55 microphytobenthos, including the diatom *Pseudo-nitzschia*, benthic infauna, and sediment toxin  
56 data were used to develop a theory for the transfer of domoic acid (DA) from the toxic diatom to  
57 the benthos in the highly productive waters of the northern Gulf of Mexico near the Mississippi  
58 River plume. Archived samples and new data were used to test the theory that DA is likely to be  
59 incorporated into benthic consumers. High spring abundances of potentially toxic *Pseudo-*  
60 *nitzschia* diatoms were simultaneously present in the surface waters, bottom waters and on the  
61 seafloor. Examination of the gut contents of a typical deposit-feeding and suspension-feeding  
62 polychaete, *Paraprionospio pinnata*, during similar periods of high *Pseudo-nitzschia* abundance  
63 in surface water indicated consumption of the diatoms. Demersal fishes, particularly Atlantic  
64 croaker, are known to consume these polychaetes, with a potential for transfer of DA to even  
65 higher trophic levels. These findings warrant a theory to be tested with further studies about the  
66 trophic linkage of a phytoplankton toxin into the benthic food web.

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70 **Keywords**

71 *Pseudo-nitzschia*, domoic acid, *Paraprionospio pinnata*, Atlantic croaker, benthic food webs,  
72 northern Gulf of Mexico

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75 **1. Introduction**

76 The northern Gulf of Mexico is a productive, but eutrophied and often hypoxic, coastal  
77 ecosystem dominated by outflows of the Mississippi and Atchafalaya rivers (Turner and  
78 Rabalais, 1994). High primary production occurs in the spring during and following high  
79 freshwater discharge and nutrient loads (Lohrenz et al., 1990; Justić et al., 2003; Lehrter et al.,  
80 2009). In the spring and late summer, chain-forming diatoms, such as *Skeletonema*, *Chaetoceros*  
81 and *Pseudo-nitzschia* dominate the surface water phytoplankton community (Dortch et al., 1997;  
82 Dortch et al., 2001; Baustian et al., 2011). The sinking phytoplankton chains, along with  
83 aggregates and fecal pellets, contribute to the flux of organic matter to the seafloor, provide a  
84 food source for benthic fauna (review by Vigilant and Silver, 2007), and fuel the microbially-  
85 mediated oxygen consumption that depletes the bottom water of oxygen (Turner and Allen,  
86 1982; Murrell and Lehrter, 2011; Turner et al., 2012).

87 *Pseudo-nitzschia* spp. have increasingly contributed to primary production in the surface  
88 waters of the northern Gulf of Mexico (Parsons et al., 2002) and worldwide (Sellner et al., 2003;  
89 Silver et al., 2010), coincident with the increasing anthropogenic nitrate-N loading to coastal  
90 waters (Hallegraeff, 1993; Parsons et al., 2002; Heisler et al., 2008). This pennate diatom is of  
91 concern to living resources, including humans, because some species of *Pseudo-nitzschia*  
92 produce the neurotoxin domoic acid (DA) that is responsible for amnesic shellfish poisoning

93 (Bates et al., 1989). High cellular and net production rates of DA are evident in cultures of  
94 *Pseudo-nitzschia* spp. collected off the Louisiana coast (Pan et al., 2001). Detectable DA  
95 concentrations have been observed in water samples from the northern Gulf of Mexico (Parsons  
96 et al., 2013; Bargu et al., 2016) and tissues of suspension-feeding gulf menhaden (Del Rio et al.,  
97 2010), and their predators, such as bottlenose dolphins (Fire et al., 2011) and sharks (Del Rio,  
98 2009).

99         Suspension-feeding bivalves are the major vector of DA to humans through consumption  
100 of shellfish. Eastern oysters (*Crassostrea virginica*) are exposed to *Pseudo-nitzschia* spp. in  
101 northern Gulf of Mexico estuaries but to date no significant measurable concentrations of DA  
102 have been observed in them (Dortch et al., 1997; Macintyre et al., 2011; O'Dea, 2012). Oysters,  
103 however, retain less DA than other bivalves through preferential rejection of toxic *Pseudo-*  
104 *nitzschia* spp. during feeding (Mafra Jr et al., 2009). Other pathways for the incorporation of DA  
105 into higher organisms are through benthic-feeding and benthopelagic-feeding flatfishes (Viligant  
106 and Silver, 2007). Those reported to feed exclusively on polychaetes incorporated more DA than  
107 those with benthopelagic-feeding habits consuming sediments with *Pseudo-nitzschia* cells, fecal  
108 pellets containing *Pseudo-nitzschia* cells, or flocculent material including *Pseudo-nitzschia* cells.  
109 The higher DA levels in those feeding on polychaetes indicate that these invertebrates may be an  
110 important vector of the toxin in benthic communities. Gut content analyses on benthic feeding  
111 fish with measurable DA concentrations identify prey primarily as clams/mussels, crustaceans,  
112 and polychaetes (Mazzillo et al., 2010). The polychaetes in these studies were not identified to  
113 species, nor were their DA levels measured or presence of *Pseudo-nitzschia* cells in the  
114 polychaete guts verified.

115           Microphytobenthos, including living *Pseudo-nitzschia* spp., are common on the inner  
116 Louisiana continental shelf sediments (Parsons et al., 2002; Grippo et al., 2010; Baustian et al.,  
117 2011, 2013). To date, no DA analyses of sinking phytoplankton detritus or surface sediments  
118 has been reported in the northern Gulf of Mexico region. The most abundant member of the  
119 benthic infaunal community, even during hypoxic events, with abundances reaching 8,000  
120 individuals m<sup>-2</sup> in late spring (Rabalais et al., 2001; Baustian and Rabalais, 2009) is the fringe-  
121 gill mudworm (*Paraprionospio pinnata*). *P. pinnata* is a suspension and surface deposit feeder  
122 (Dauer et al., 1981) and, thus, may consume *Pseudo-nitzschia* from surface sediments and act as  
123 a vector of DA to higher trophic levels, including the highly abundant demersal predators such as  
124 Atlantic croaker (*Micropogonias undulatus*) (Chesney and Baltz, 2001) that are known to  
125 opportunistically feed upon these polychaetes (Baustian et al., 2009).

126           Archived samples were used to identify the linkages among surface water *Pseudo-*  
127 *nitzschia* populations, *Pseudo-nitzschia* cells in bottom waters and sediments, and eventually the  
128 presence of *Pseudo-nitzschia* cells in the gut contents of the benthic polychaete, *Paraprionospio*  
129 *pinnata*, as an example of a benthic organisms that could serve as a vector for transferring DA to  
130 higher trophic levels. Not all aspects of the surface water *Pseudo-nitzschia* production to benthic  
131 polychaete to demersal fishes transfer of DA were available, and no DA assays of benthic fauna  
132 were available, but enough evidence led us to support a polychaete source of DA to the benthic  
133 food web on this continental shelf.

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## 135   **2. Materials and methods**

136 Archived phytoplankton, microphytobenthos, polychaete infaunal, and sediment samples  
137 collected from prior studies (Baustian and Rabalais, 2009; Baustian et al., 2011, 2013) were used  
138 to test a theory for trophic transfer of DA into the benthic food web. Not all three community  
139 types were sampled at the same time, but data sets were examined in groups (phytoplankton vs.  
140 microphytobenthos; phytoplankton vs. polychaete gut contents; microphytobenthos vs. DA) for  
141 temporal variability of *Pseudo-nitzschia* and its toxin.

## 142 2.1 Phytoplankton and microphytobenthos

143 Phytoplankton and microphytobenthos were sampled from the northern Gulf of Mexico inner  
144 continental shelf on a transect south of Terrebonne Bay, LA about 100 km west of the  
145 Mississippi River Delta (Fig. 1) in an area that is characteristically hypoxic in the bottom water  
146 during summer (Rabalais et al., 2007; Baustian et al., 2011) and where high abundances of  
147 *Pseudo-nitzschia* occur in spring (Dortch et al., 1997; Parsons et al., 2013; Barga et al., 2016).  
148 Phytoplankton in surface water samples and microphytobenthos in the top 0.5 cm of surficial  
149 sediments from box cores were enumerated at stations C4 (~14 m depth, 28:57.00' N, 90:31.46'  
150 W), C6B (~20 m depth, 28:52.18' N, 90:28.04' W) and C8 (~23 m depth, 28:47.30' N, 90:16.60'  
151 W) at approximately bimonthly intervals from July 2006 to July 2008 (Fig. 1). Epifluorescence  
152 microscopy (Olympus BH-2-RFCA) with blue and green excitation with 0.03% proflavin vital  
153 stained slides were used to highlight the chloroplasts and nuclei for identification of viable cells  
154 (Baustian et al., 2011). Archived surface phytoplankton samples that were collected monthly  
155 from September 2003 to February 2004 at stations C6B and C6C (within 1 km of each other, ~  
156 20 m depth) from March to October 2004 (see Fig. 1) were used to enumerate *Pseudo-nitzschia*  
157 cells for comparison to the polychaete gut analyses.



## 158 2.2 Scanning electron microscopy

159 Scanning electron microscopy (SEM) was used to identify the species of *Pseudo-nitzschia* in the  
160 2006 to 2008 surface water, bottom water, and sediment samples if epifluorescence microscopy  
161 methodologies observed high densities of *Pseudo-nitzschia* cells. Samples were concentrated  
162 onto 1.2- $\mu$ m pore size isopore polycarbonate membrane filters (Millipore) (Bargu et al., 2008).  
163 Salt was removed by rinsing with DI water under low vacuum pressure (150 mm Hg). To  
164 remove organic material, 2-3 drops of saturated KMnO<sub>4</sub> were added until the filters were  
165 covered and allowed to digest for 30 min. Samples were then treated with 3 ml of 12N HCl until  
166 the color became clear or held for 60 minutes to complete the oxidation process. Cleaned  
167 samples were rinsed twice with DI water and filters were mounted onto SEM stubs with double-  
168 sided tape. Mounted filters were air-dried in a desiccator for 24 h and sputter coated with gold  
169 palladium. All SEM micrographs were obtained with a Cambridge Stereoscan 260 scanning  
170 electron 22 microscope at 10 kV.

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## 172 2.3 Polychaete gut contents

173 Archived *P. pinnata* (macrobenthos stored in ethanol) were collected monthly from September  
174 2003 to October 2004 at station C6B (Baustian and Rabalais, 2009) for gut content analyses that  
175 were consistent in time to surface phytoplankton samples. This station was examined for  
176 microphytobenthos by Baustian et al. (2011) and is also part of a long-term phytoplankton  
177 community monitoring data base (Dortch et al., 1997; Parsons et al., 2015,  
178 <http://dx.doi.org/10.7266/N7PK0D3S>).

179 Two individuals of *P. pinnata* were selected as replicates from each monthly  
180 macrobenthos sample based on two criteria: (1) an intact body and (2) the polychaetes were  
181 similar in length. Only one intact *P. pinnata* was available for February 2004. The polychaetes  
182 were dissolved using a method adapted from Del Rio et al. (2010) and Bargu et al. (2008) that  
183 involved a strong oxidizer (KMnO<sub>4</sub>) and acid (HCl). The number of treatments depended on the  
184 length of the polychaete, with small polychaetes requiring two treatments and the large  
185 polychaetes requiring up to five treatments. The digestion process dissolved the organic material  
186 but not the silica diatom frustules in the gut. Light microscopy (Olympus BH-2-RFCA) at 400x  
187 magnification was used to identify diatoms from the gut content digestions. The entire area under  
188 the cover slip was scanned for whole and partial *Pseudo-nitzschia* valves and were counted  
189 according to Schrader and Gersonde (1978) that required presence of at least half of a valve to be  
190 enumerated. Units are mean number of *Pseudo-nitzschia* valves (observed from two  
191 polychaetes).

#### 192 2.4 Sediment DA analysis

193 Archived sediment samples (depths of 2.5 – 3 cm from stations C6B and C8 from 21 March  
194 2007 and 12 May 2008) were stored in -80°C until analyzed for DA in June 2016 following  
195 similar methods of Bargu et al. (2016). Aliquots of up to 3 g of the sediment were extracted  
196 applying a 1:4 ratio of sediment to 50% aqueous MeOH. Samples were sonicated on ice for 2  
197 min, centrifuged for 10 min at 5000 RPM, and the supernatant was filtered (0.22 µm) into clean  
198 centrifuge tubes. A competitive enzyme linked immunosorbent assay (ASP Direct cELISA Kit,  
199 Biosense Laboratories AS, Norway) was used to quantify DA concentrations. Each sample was  
200 run in duplicate at multiple dilutions according to the manufacturer's specifications. The

201 absorbance data for each sample were collected using a micro-plate spectrophotometer set at a  
202 wavelength of 450 nm. The sediment from which DA was extracted was dried and weighed to  
203 obtain concentrations of DA in ng g dry sed<sup>-1</sup>.

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### 205 3. Results

#### 206 3.1 Seasonal and spatial distribution of *Pseudo-nitzschia*

207 *Pseudo-nitzschia* spp. from the June 2006 to July 2008 samples at all stations ranged from non-  
208 detectable to  $4 \times 10^6$  cells l<sup>-1</sup> in surface water, from non-detectable to  $1.2 \times 10^6$  cells l<sup>-1</sup> in the  
209 bottom water and from non-detectable to  $4 \times 10^2$  cells g dry sed<sup>-1</sup> on the sediment surface (Fig.  
210 2). Peak surface-water abundances of cells were evident in the spring of 2007 and 2008 at all  
211 stations (Fig. 2A). *Pseudo-nitzschia* spp. contributed 68%, 79%, and 86% to the total cell density  
212 of the surface waters in May 2007 at stations C4, C6B, and C8, respectively.

213 Spring cell density peaks were present in the bottom water for most of the stations in 2007 and  
214 2008, with the peaks corresponding to the surface-water cell density peaks (Fig. 2B). Bottom-  
215 water *Pseudo-nitzschia* densities at stations C4 and C8 were also highest in May 2007 and  
216 contributed 70%, 54%, and 73% of the May 2007 bottom-water total cell density at stations C4,  
217 C6B, and C8, respectively.

218 *Pseudo-nitzschia* cells were observed on the sediment surface on three occasions, all during the  
219 spring months and only at two stations (C4 and C6B) from 2006 to 2008 (Fig. 2C). Observations  
220 with light and epifluorescence microscopy indicated they were still in chain forms and had  
221 visible chloroplasts, thus considered viable. Sediment *Pseudo-nitzschia* cells were present at

222 station C6B in March 2007 with  $3.7 \times 10^2$  cells g dry sed<sup>-1</sup>, in May 2007 with  $0.15 \times 10^2$  cells g  
223 dry sed<sup>-1</sup>, and at station C4 in May 2008 with  $1.6 \times 10^2$  cells g dry sed<sup>-1</sup>. The percent  
224 contributions of *Pseudo-nitzschia* spp. to the total microphytobenthos were 7% (C6B March  
225 2007), 0.1% (C6B May 2007), and 4% (C4 May 2008). No *Pseudo-nitzschia* cells were detected  
226 on the sediment surface at the deepest station (C8). The percent contribution of *Pseudo-nitzschia*  
227 spp. to the microphytobenthos community was minimal (0.01%) across the continental shelf  
228 during mid-summer of 2006, 2007, and 2008 (Baustian et al., 2013).

229 Surface-water data concurrent with the polychaete collections indicated peaks of *Pseudo-*  
230 *nitzschia* spp. in spring 2004 (Dortch et al. unpubl. data), with the highest densities reaching  $\sim 6$   
231  $\times 10^5$  cells l<sup>-1</sup> in March 2004 (Fig. 3).

### 232 3.2 SEM identifications

233 The SEM verified that most of the *Pseudo-nitzschia* cells in the 2006 to 2008 surface water,  
234 bottom water, and sediment surface samples belonged to the *P. pseudodelicatissima* complex  
235 with the two common species being *P. calliantha* and *P. pseudodelicatissima*. *P. calliantha* was  
236 identified in the surface (Fig. 4A) and bottom water of station C4 in May 2007 and in the bottom  
237 water of C4 in May 2008. *P. pseudodelicatissima* was found in the surface water (Fig. 4A) and  
238 bottom water at station C4 in May 2007. Valves belonging to the *P. pseudodelicatissima*  
239 complex were common in sediments from station C6B in March 2007, and were also identified  
240 from sediments at station C4 in May 2008 (Fig. 4C and 4D). Some of the *Pseudo-nitzschia* cells  
241 from sediments at station C6B in May 2007 did not digest completely, which prevented  
242 identification to the species level (Fig. 4B).

### 243 3.3 Polychaete gut contents

244 *Pseudo-nitzschia* were found in the guts of *P. pinnata* and ranged from undetected to an average  
245 of six valves per monthly sample of two digested worms (Fig. 3). Approximately one third of  
246 *Pseudo-nitzschia* valves were intact and the rest were fragmented. The occurrence of *Pseudo-*  
247 *nitzschia* valves in *P. pinnata* sometimes paralleled the peak abundance of *Pseudo-nitzschia* cells  
248 in the surface waters (Fig. 3) but the correlation with surface water abundance and gut contents  
249 was weak ( $r^2 = 0.01$ ,  $p = 0.69$ ,  $n = 14$ ). *Pseudo-nitzschia* spp. comprised an average 15% of total  
250 water column diatom abundance for the study period, but they represented only 0 to 7% of the  
251 total diatoms (centric, pennate, and *Pseudo-nitzschia* spp.) in the polychaete guts.

### 252 3.4 Sediment DA analysis

253 Domoic acid concentrations were below the detection limit ( $< 0.17$  ng g dry sed<sup>-1</sup>) from the C8  
254 sediment sample in 21 March 2007 and were above detection limit ( $> 0.17$  ng g dry sed<sup>-1</sup>) in  
255 sediment samples at station C6B in both 21 March 2007 and 12 May 2008 (Table 1). The DA  
256 concentrations were still detected in sediments from station C6B in May 2008 even though the  
257 sediment surface at both stations did not have *Pseudo-nitzschia* cells observed (Table 1). No  
258 sediment samples were available from station C4.

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## 260 **Discussion**

261 Benthic-pelagic coupling of *Pseudo-nitzschia* spp. through ingestion by the polychaete  
262 *Paraprionospio pinnata*, provides a likely mechanism for transfer of the diatom toxin, DA, into  
263 the benthic food web of the northern Gulf of Mexico. Peak *Pseudo-nitzschia* abundance in the

264 surface water in spring 2007 were followed by peaks of viable *Pseudo-nitzschia* cells in the  
265 bottom water and on the sediment surface. This pattern indicates that *Pseudo-nitzschia* cells were  
266 sinking to the seafloor at stations C4 and C6B in depths of 14 and 20 m. However, the highest  
267 sediment abundance of *Pseudo-nitzschia* spp. in 2007 preceded the surface peak abundance at  
268 the same site by two months, indicating the coarse temporal sampling regime may have missed a  
269 prior surface *Pseudo-nitzschia* bloom. Alternatively, if *Pseudo-nitzschia* spp. have resting stages,  
270 they may act as a reservoir or inoculum for the water column above them (Lelong et al., 2012).  
271 *Pseudo-nitzschia* cells were not present on the surface sediments during the spring at station C8  
272 (23 m water depth), even though the highest surface-water density of *Pseudo-nitzschia* spp. was  
273 at station C8 in May 2007. Station C8 (with sandier sediment than C4 and C6B) did not become  
274 hypoxic during the summer of 2007 and 2008 (Baustian et al., 2011), which may indicate that the  
275 carbon flux, including cells of *Pseudo-nitzschia* spp. may have been low at this depth. In  
276 addition, few to no *Pseudo-nitzschia* cells were observed on the sediment surface during mid to  
277 late July 2006, 2007 and 2008 along the Louisiana continental shelf suggesting that *Pseudo-*  
278 *nitzschia* spp. were more common on the sediment surface during periods of high spring surface  
279 production.

280           The common *Pseudo-nitzschia* species found on the sediment surface, based on SEM  
281 analyses, were *P. calliantha* and *P. pseudodelicatissima*, which are confirmed DA producers in  
282 the northern Gulf of Mexico (Parsons et al., 1999; Pan et al., 2001; Del Rio et al., 2010). Thus,  
283 sinking, viable cells belonging to the *P. pseudodelicatissima* complex have the potential to  
284 transport DA to benthic communities. If the toxic cells of *Pseudo-nitzschia* spp. become lysed,  
285 the dissolved form of DA could remain in the sediment for some time by binding to silts and

286 clays (Burns and Ferry, 2007), which are common in the muddy sediments of stations C4 and  
287 C6B (Baustian et al., 2011). There is uncertainty in the varying toxin levels in the viable and  
288 senescent cells and the amount of time that DA contamination persists in the sediment, but  
289 sediment DA was present at station C6B and intact cells were found in the guts of  
290 *Paraprionospio pinnata*.

291         Surface deposit-feeding and suspension-feeding polychaetes, such as *P. pinnata*, are the  
292 most abundant benthic infauna in this area (Baustian and Rabalais, 2009) and are a likely vector  
293 to transport diatom toxins to upper trophic levels of the benthic food web in northern Gulf of  
294 Mexico as well as elsewhere (Vigilant and Silver, 2007). The gut content analyses of *P. pinnata*  
295 from our study area included cells of *Pseudo-nitzschia*, but the highest abundances were not  
296 during the spring months of 2004 as expected, which could simply be due to the low number of  
297 polychaetes that were analyzed. In addition, the spring 2004 surface water *Pseudo-nitzschia* spp.  
298 abundances ( $\sim 6 \times 10^5$  cells  $l^{-1}$ ) were not as high as the spring 2007 surface water abundances ( $\sim 3$   
299  $\times 10^6$  cells  $l^{-1}$ ) that represent bloom conditions. With low surface-water cell abundances, the  
300 probability of these cells fluxing to the sediment and being incorporated into polychaetes could  
301 be low. These polychaetes can reject food brought to their pharynx by their palps, and may reject  
302 up to 50% of the food collected (Dauer, 1985). For example, fecal pellets and cyanobacteria were  
303 never selected during deposit feeding and flocculent material was commonly ingested by  
304 specimens from Chesapeake Bay (Dauer, 1985). *Pseudo-nitzschia* are likely present in the  
305 flocculent material of the northern Gulf of Mexico, which is probably the source of consumed  
306 cells by polychaetes.

307           One-third of the *Pseudo-nitzschia* valves were intact in the guts of *P. pinnata*, indicating  
308 they may have been viable and contained DA. It is not known what the rate of deterioration of  
309 the *Pseudo-nitzschia* frustule after cell senescence is or how long ingested viable valves persist.  
310 However, it is known that intact *Pseudo-nitzschia* frustules are identifiable in at least 50-year old  
311 sediment samples (Parsons et al., 2002) and that dissolution is unlikely for frustules retained in  
312 historic sediments. Broken frustules on the sediment surface could directly contribute to the  
313 sediment toxin pool and contribute to accumulation through trophic transfer. Fish, sediment,  
314 snails, and dolphins were detected with DA in the eastern Gulf of Mexico and DA trophic  
315 transfer was likely occurring in that region (Twiner et al., 2011). Demersal predators of the  
316 northern Gulf of Mexico, such as penaeid shrimp, blue crabs, Atlantic croaker, and cownose rays  
317 are known to prey upon polychaetes and bivalves (Baustian et al., 2009; Craig et al., 2010) and  
318 could be affected by toxins that commonly occur within three trophic levels (Trainer et al.,  
319 2012), for example: DA in *Pseudo-nitzschia* → polychaete → Atlantic croaker. The presence of  
320 *Pseudo-nitzschia* spp. in the gut contents of *P. pinnata* supports the theory that these worms and  
321 other infauna are potential vectors of DA to higher trophic levels as in other benthic  
322 communities. A finer temporal approach using our techniques coupled with particle traps and  
323 demersal fish assessments would better define the coupling of DA toxins from spring blooms of  
324 the *Pseudo-nitzschia pseudodelicatissima* complex to benthic consumers and further transfer  
325 through the food web.

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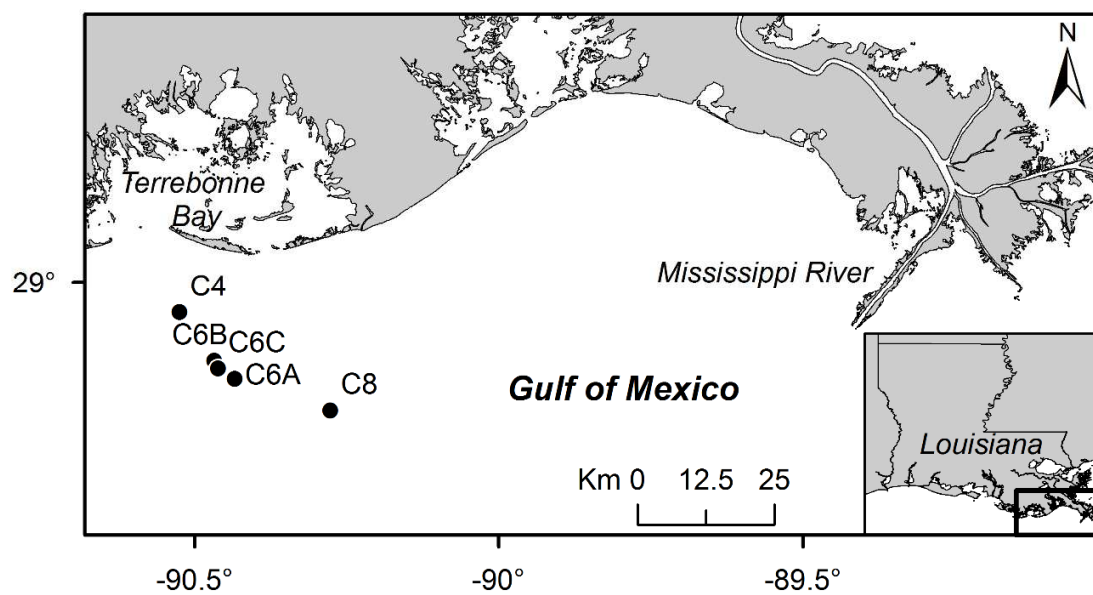


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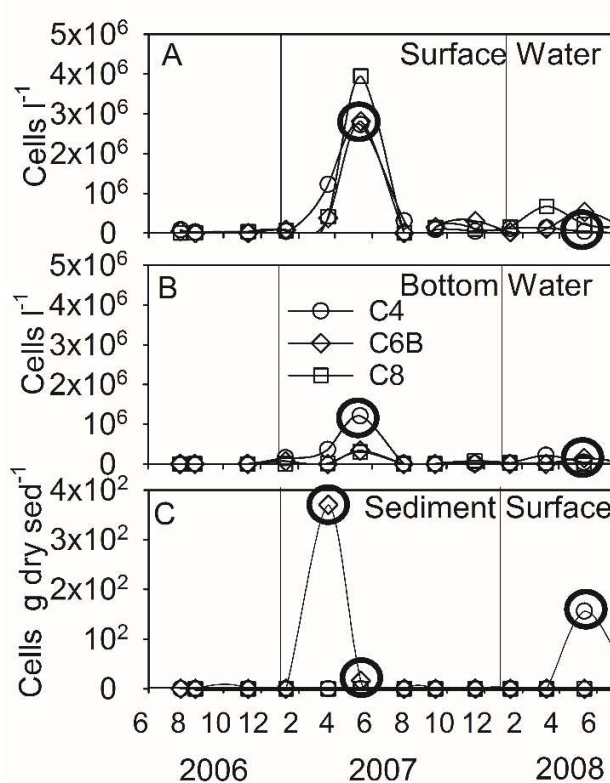
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487 Figures



488 **Fig. 1.** Station C4, C6A, C6B, C6C, and C8 locations west of the Mississippi River in the  
489 northern Gulf of Mexico.

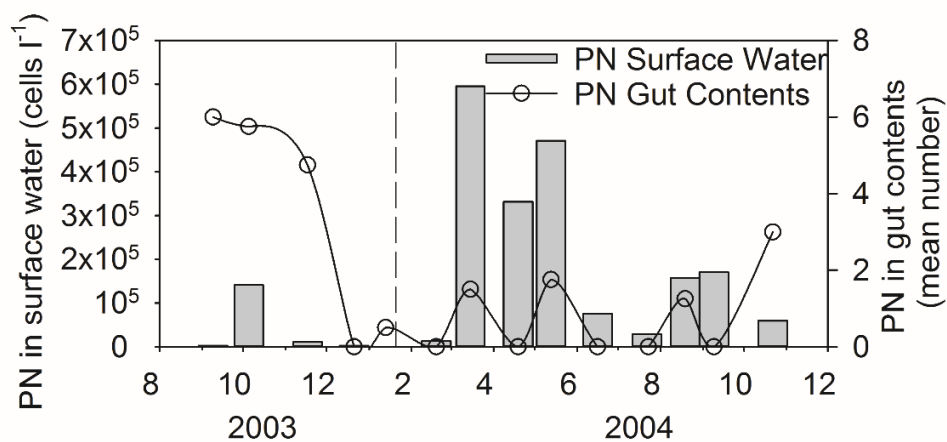
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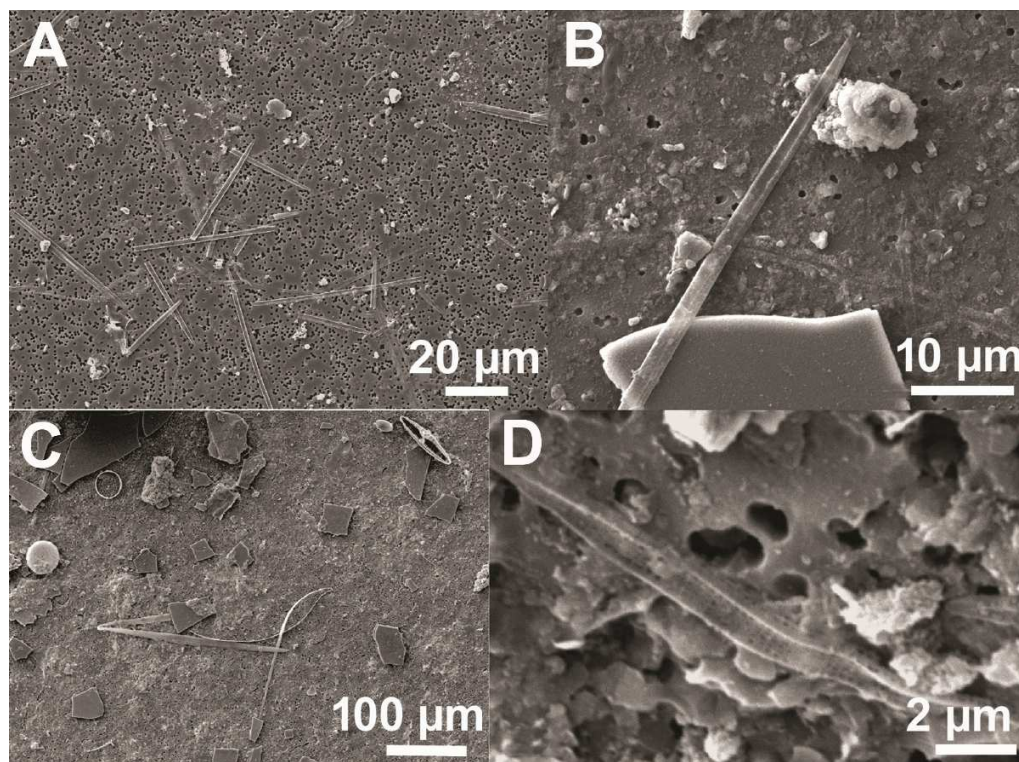
494 **Fig. 2.** *Pseudo-nitzschia* spp. cells in the A) surface water (n= 39), B) bottom water (n= 39) and  
 495 C) sediment surface (n= 38) at stations C4, C6B and C8 from June 2006 to July 2008. Circled  
 496 points indicate scanning electron microscopy (SEM) images were taken of surface water (station  
 497 C6B in May 2007 and station C4 in May 2008), bottom water (station C4 in May 2007 and  
 498 station C6B in May 2008) and sediment surface samples (station C6B in March 2007 and May  
 499 2007, station C4 in May 2008).



500

501 **Fig. 3.** *Pseudo-nitzschia* spp. (PN) abundances in surface water samples (data from Dortch et al.  
 502 unpubl. data) and in gut contents of polychaete *P. pinnata* (mean of two) at station C6B from  
 503 August 2003 to December 2004. Surface water samples were collected at station C6B until  
 504 February 2004 (dashed line) when the sampling was relocated a short distance (within 1 km east)  
 505 away to station C6C (see Fig. 1 for station locations).

506



507

508 **Fig. 4.** Scanning electron microscopy images of A) *P. pseudodelicatissima* complex (*P.*  
 509 *calliantha* and *P. pseudodelicatissima* with presence of single row of poroids within the striae, a  
 510 central nodulus is present in both species) from the surface water of station C4 in May 2007, B)  
 511 unidentifiable *Pseudo-nitzschia* spp. from the sediment surface of station C6B in May 2007, C)  
 512 valve of unidentifiable *Pseudo-nitzschia* spp. from the sediment surface at station C6B in March  
 513 2007 (note other pennate and centric diatoms) and D) valve of *P. cf. pseudodelicatissima*  
 514 complex on the sediment surface at station C4 in May 2008.

515

516

517 **Table 1.** Presence of *Pseudo-nitzschia* cells (PN, cells g dry sed.<sup>-1</sup>) and domoic acid  
 518 concentrations (DA, ng g dry sed.<sup>-1</sup>) from sediment samples (depths of 2.5 to 3 cm) collected at  
 519 stations C6B and C8 in spring months of 2007 and 2008. No archived sediment samples were  
 520 available from station C4. DA symbols of “-” indicate sediment samples were analyzed but  
 521 concentrations were below the detection limit (< 0.17 ng g dry sed<sup>-1</sup>), “+” indicates sediment  
 522 samples were analyzed and were above detection limit (> 0.17 ng g dry sed<sup>-1</sup>), ND = no data.

Sample Date	C6B		C8	
	PN	DA	PN	DA
3/21/2007	370	+	0	-
5/13/2007	16	ND	0	ND
5/12/2008	0	+	0	ND

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524