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An assessment of *Pseudo-nitzschia* population dynamics and domoic acid production in coastal Louisiana

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ABSTRACT

Over 1200 samples were collected from Louisiana estuarine and coastal shelf waters between 1989 and 2002, and analyzed to examine the population dynamics of *Pseudo-nitzschia* and to assess the potential threat posed by domoic acid (DA), a potent neurotoxin produced by some members within this toxigenic diatom genus. Results demonstrated that three species in this region (*Pseudo-nitzschia multiseries*, *P. pseudodelicatissima* complex, *P. delicatissima*) produce DA, and that particulate toxin levels were highest (up to 3.05 $\mu g\ L^{-1}$) during the spring bloom, while cellular concentrations were highest in the winter/early spring when *P. multiseries* was most abundant (up to 30 pg $cell^{-1}$). These particulate toxin levels are comparable to those seen in other regions (e.g., United States west coast) where DA poisoning events have occurred in the past. *Pseudo-nitzschia* were most abundant under dissolved inorganic nitrogen-replete conditions coupled with lower silicate and/or phosphate concentrations, and in the early spring months when temperatures were cooler. *Pseudo-nitzschia* were occasionally well-represented in the phytoplankton assemblage ($\geq 10^6$ cells L^{-1} in 14% of samples, over 50% of total phytoplankton in 5% of samples), indicating that planktivores (e.g., Gulf menhaden, *Brevoortia patronus*) may have little choice but to consume *Pseudo-nitzschia* cells, thereby providing potential vectors for DA transfer to higher trophic levels. By comparison, eastern oysters (*Crassostrea virginica*) present in estuarine waters may be more exposed to this toxin when *Pseudo-nitzschia* cells are part of a mixed assemblage, reducing selective grazing by these bivalves. *C. virginica* may thus represent the most effective vector for DA exposure in humans.

1. Introduction

Pseudo-nitzschia is a ubiquitous genus of diatoms (Bacillariophyceae) that contains some species capable of producing the neurotoxin, domoic acid (DA). *Pseudo-nitzschia* was first identified as a harmful algal bloom genus following a shellfish poisoning event in 1987, when several people died and over one hundred became ill from eating blue mussels (*Mytilus edulis*) contaminated with DA produced by *Pseudo-nitzschia multiseries* (=*Nitzschia multiseries*; *Nitzschia pungens* forma *multiseries* (Hasle, 1994, 1995)) harvested from Prince Edward Island, Canada (Bates et al., 1989). Since then, DA produced by *Pseudo-nitzschia* has been implicated in the illness and death of many marine mammals and birds (reviewed in Trainer et al., 2012). DA is routinely monitored for and detected in seafood from coastal waters of Washington and California, USA (Trainer and Suddleson, 2005; Langlois, 2007).

The threats posed by *Pseudo-nitzschia* led to wide-ranging research efforts to determine which species of *Pseudo-nitzschia* are toxigenic, under what conditions these species bloom and/or produce DA, and potential vectors of the transfer of this toxin to higher trophic levels. Results of these studies (summarized in Lelong et al., 2012 and Trainer et al., 2010) have identified 14 species of *Pseudo-nitzschia* currently known to be capable of DA production. Of these 14, only seven have been subjected to culture-based studies to determine how environmental factors affect toxin production. Generally speaking, the toxigenic members of *Pseudo-nitzschia* tend to produce more toxin when silica, phosphate, and/or iron are limiting, when nitrogen is plentiful, and at higher light and salinities (summarized in Lelong et al., 2012). These conditions are also conducive for *Pseudo-nitzschia* to bloom (although nutrients cannot be limiting prior to bloom formation), and occur regularly in regions where upwelling is common (e.g., coastal California; Lane et al., 2009; Juan de Fuca eddy; Trainer et al., 2002) or where riverine inputs are significant (e.g., northern Gulf of Mexico; Dortch et al., 1997; Aegean Sea; Spatharis et al., 2007).

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The initial case of DA poisoning in 1987 was caused by the consumption of blue mussels (*Mytilus edulis*; Wright et al., 1989). Anchovies were the vector for DA poisoning of marine mammals and seabirds in California (e.g., Work et al., 1993; Lefebvre et al., 1999). DA was also detected in razor clams from coastal Washington and Oregon (Wekell et al., 1994), which in turn were a vector for contamination of Dungeness crabs (Wekell et al., 1994). These examples demonstrate that filter-feeding shellfish and clupeoid fishes are among the primary vectors for DA transfer to higher trophic levels (reviewed in Trainer et al., 2012). What is unclear, however, is why these vectors are effective in some regions where toxigenic *Pseudo-nitzschia* blooms occur, but not in others such as coastal Louisiana.

The coastal waters of Louisiana comprise a highly productive marine ecosystem, in which Gulf menhaden (*Brevoortia patronus*) support the second largest U.S. fishery by weight and penaeid shrimps support the fifth largest fishery by value ($300–400 million per year; de Mutsert et al., 2008). Much of this productivity is supported by the high nutrient load supplied by the Mississippi River, with a drainage basin comprising 41% of the contiguous United States and small portions of Canada, including the nation's "breadbasket" of intensively farmed and fertilized mid-western lands. While the high nutrient loads support productive fisheries, they are also causing an overproduction of organic matter leading to severe hypoxia on the Louisiana shelf (Rabalais et al., 2002a,b). Scientific efforts have been ongoing since the 1980s to study the nature of this primary production (predominantly diatoms; Turner et al., 1998; Dortch et al., 2001; Wawrik and Paul, 2004), including the processes involved in the transfer of this biomass to higher trophic levels in the pelagic zone (i.e., grazing processes; Dagg, 1995; Turner et al., 1998) and to the benthos (e.g., Rabalais et al., 1996, 2002a,b, 2007).

Phytoplankton blooms commonly occur downstream of the Mississippi River during the high riverine discharges associated with the annual spring flood (Lohrenz et al., 1997) and *Pseudo-nitzschia* is a common member of the blooming assemblage (Dortch et al., 1997). Concerns that blooms of toxigenic *Pseudo-nitzschia* could lead to DA poisoning events in the region led to further research in which several toxigenic species were identified, including *Pseudo-nitzschia calliantha*, *P. multiseries*, and *P.* c.f. *pseudodelicatissima* (Parsons et al., 1998, 1999; Pan et al., 2001; Del Rio et al., 2010), and DA was detected and quantified in water samples and *Pseudo-nitzschia* cultures (Parsons et al., 1999; Pan et al., 2001; Del Rio et al., 2010).

Pseudo-nitzschia populations were shown to be euryhaline (Dortch et al., 1997; Thessen et al., 2005), leading to the possibility of eastern oysters (*Crassostrea virginica*) serving as a potential vector for DA exposure in humans. Thessen et al. (2010) demonstrated that whereas oysters feed more readily on smaller diatoms than on long, pointy cells such as *Pseudo-nitzschia*, there was no evidence that oysters avoided *Pseudo-nitzschia* due to their toxin content. Thessen et al. (2005) found that *Pseudo-nitzschia multiseries* could grow at salinities as low as 10 (albeit at a much slower rate), whereas Doucette et al. (2008) reported that DA production was substantially lower at salinities of 10 and 20 versus 30 and 40, which they attributed to a need to divert energy resources to osmoregulation rather than photosynthesis and toxin production. Del Rio et al. (2010) detected DA in estuarine water samples containing *Pseudo-nitzschia* cells and in Gulf menhaden, but speculated that the low abundance of *Pseudo-nitzschia* in the estuary (Terrebonne Bay) served to prevent any significant transfer of toxin to higher trophic levels.

Pseudo-nitzschia are present in estuarine waters of Louisiana (Dortch et al., 1997; Thessen et al., 2005; Del Rio et al., 2010), but to date have caused no known cases of DA poisoning. *Pseudo-nitzschia* prevalence in the shelf waters of Louisiana (Dortch et al., 1997) and the large spring blooms in this region could provide a source from which significant amounts of this toxin are transferred to higher trophic levels in coastal food webs, primarily through grazing by clupeoid fishes and zooplankton, and advection of shelf waters into estuaries where oysters reside. The purpose of this study, therefore, was to determine the conditions that lead to high *Pseudo-nitzschia* biomass and cellular DA content in coastal versus estuarine environments, which could result in such a trophic transfer of this potent neurotoxin.

2. Methods

2.1. Sample collection and preparation

Surface (0–1 m depth) whole water grab and net tow samples (30 cm diameter, 2 m length, 35 μm Nitex® mesh) were collected between 1989 and 2002 along the A, A′, B, C (including an additional estuarine site – Midbay), D, D′, E, F, and K transects on the Louisiana shelf (Fig. 1). The coastal C-transect stations (particularly C1, C6B, and C9) were sampled most frequently, generally on a monthly basis (as was the estuarine station, Midbay). An additional estuarine site (T3) was sampled weekly between 1993 and 1998 (Table 1), during which water samples were collected with a hand-operated peristaltic pump. Whole water and net tow samples were prepared for microscopy following Dortch et al. (1997), in which aliquots were preserved in 0.5% glutaraldehyde, stained with 0.03% proflavine hemisulfate, and size-fractionated through 25 mm diameter polycarbonate 8 μm (net tow and water) and 3 μm (water only) pore-size filters. Preliminary analyses indicated that all of the *Pseudo-nitzschia* cells were accounted for in the 8 and 3 μm filter accounts (i.e., no cells passed through the 3 μm filter), with an average of 86% of the cells collected on the 8 μm filter (n = 907). While *Pseudo-nitzschia* cells and chains more than likely passed through the net tow mesh, the need to concentrate cells and remove sediments to facilitate species identifications and toxin analyses led to this choice for sample collection. As cell concentrations were based on water samples rather than net tow samples (see below), and species identifications and toxin analyses were based on the actual number of cells collected, the potential loss was deemed of little consequence.

2.2. Pseudo-nitzschia *enumeration and abundance*

Pseudo-nitzschia cells were enumerated on the filters using an Olympus BH2-RFCA epifluorescence microscope with blue and green excitation light, and transmitted light when necessary. Abundance of *Pseudo-nitzschia* (cells L^{-1}) was then calculated from these counts, based on the number of fields counted per filter and volume of water filtered. The net tow abundance values for *Pseudo-nitzschia* were used for cellular DA calculations (see Section 2.5). The absolute abundance of *Pseudo-nitzschia* in the whole water

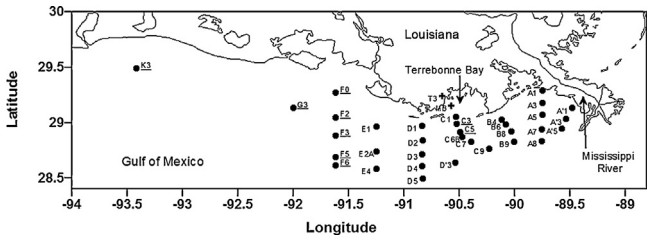

Fig. 1. Map of coastal Louisiana displaying the coastal (circles) and estuarine (crosses) stations where at least five samples were collected between 1989 and 2002 for analysis during this study (water grab only stations are underlined; water grab and net tow samples were collected at all of the other stations).