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Gulf menhaden (*Brevoortia patronus*): A potential vector of domoic acid in coastal Louisiana food webs

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ABSTRACT

Harmful algal blooms are an increasing problem for coastal waters world-wide. The presence of the toxigenic diatom genus *Pseudo-nitzschia* is of concern in the Gulf of Mexico, due to the potential for several species in this genus to produce the neurotoxin domoic acid (DA). Louisiana coastal waters are of particular interest due to the presence of both toxin-producing species of *Pseudo-nitzschia* and abundant potential vectors. While trophic transfer of DA to consumers has repeatedly occurred along the California coast, little is known about trophic transfer of recently detected DA in the Gulf of Mexico. In this study, the presence of DA was investigated in filter-feeding gulf menhaden (*Brevoortia patronus*) and in seawater where high abundances of these fish reside. *Pseudo-nitzschia* presence and enumeration was determined using light microscopy, species identification in seawater and gulf menhaden gut contents was conducted with transmission electron microscopy (TEM), and DA quantification in corresponding seawater and tissue samples was determined by competitive enzyme-linked immunosorbent assay (cELISA). Examination of the phytoplankton revealed the presence of four species of *Pseudo-nitzschia*: *P. calliantha* for the first time, *P. pseudodelicatissima*, *P. pungens*, and *P. americana*, with *P. calliantha* as the dominant *Pseudo-nitzschia* species. Low levels of DA were detected in both seawater and fish samples, with a significant correlation between the two ($n = 22$, $p = 0.043$). Thus, for the first time in the Gulf of Mexico, a potential vector of DA has been identified, revealing the possibility of DA contamination in coastal Louisiana food webs.

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1. Introduction

Harmful algal blooms (HABs) are accumulations of algal biomass to "sufficient" levels for negative impacts to occur in the environment through their morphology, sheer biomass, or toxin production (Hallegraeff, 1993; Smayda, 1997; Glibert et al., 2005a). Incidents of HABs have increased with escalating eutrophication of coastal water bodies around the world (e.g. Glibert et al., 2005b). The negative consequences of a HAB pose a significant threat to the environment and human health which can damage ecosystem function (Glibert et al., 2005a). Despite the threat, the link between HABs and upper trophic level consumers has yet to be adequately investigated for many coastal systems.

Due to increasing eutrophication from Mississippi River discharge, HAB species pose a threat to Louisiana coastal waters (Turner and Rabalais, 1994). Exacerbating this threat is the abundance of potential food web vectors in coastal Louisiana including oysters and planktivorous fishes. These conditions create a region that has a high potential for the rapid transfer of algal toxins to upper trophic level consumers.

The dominant harmful algal group in coastal Louisiana is the pennate diatom genus *Pseudo-nitzschia*, of which some species are capable of producing a powerful neurotoxin called domoic acid (DA). Although these diatoms have been identified in the northern Gulf of Mexico since the 1910s, abundances of *Pseudo-nitzschia* have been increasing since the 1950s (Parsons et al., 2002). Currently, these diatoms are frequently detected at bloom concentrations (e.g. Dortch et al., 1997). Previous examinations of the *Pseudo-nitzschia* community in Louisiana coastal waters revealed six different species with two confirmed DA producers, *Pseudo-nitzschia multiseries* and *P. pseudodelicatissima*, two potentially toxic species, *P. delicatissima* and *P. pungens*, and two non-toxic species, *P. subfraudulenta* and *P. americana*, in abundances that have been documented to reach intense bloom concentrations of 10^8 cells l^{-1} on the Louisiana continental shelf and 10^6 cells l^{-1} in Terrebonne Bay, an inshore Louisiana estuary (Dortch et al., 1997; Parsons et al., 1999; Pan et al., 2001). Although presence of *Pseudo-nitzschia* has been well documented in Louisiana's coastal

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waters, no studies have characterized the extent of its role in marine food webs of the northern Gulf of Mexico.

DA has been responsible for both human and marine animal deaths around the world (Bates et al., 1989; Wright et al., 1989; Perl et al., 1990; Work et al., 1993; Scholin et al., 2000; Fire et al., 2009). Several major marine animal mass mortality events have occurred on the west coast of the U.S. including hundreds of brown pelicans (*Pelecanus occidentalis*), Brant's cormorants (*Phalacrocorax penicillatus*), and California sea lions (*Zalophus californianus*) (Work et al., 1993; Scholin et al., 2000). In these instances, the main vector of DA was identified as a planktivorous fish, the northern anchovy (*Engraulis mordax*) (Work et al., 1993; Lefebvre et al., 1999, 2002; Scholin et al., 2000). Many other DA vectors including krill (*Euphausia pacifica* and *Thysanoessa spinifera*), market squid (*Loligo opalescens*), razor clams (*Siliqua patula*), and blue mussels (*Mytilus edulis*) have also been identified (Bates et al., 1989; Wright et al., 1989; Wekell et al., 1994; Bargu et al., 2002, 2003, 2008), but planktivorous fishes represent the most effective pathway for toxin transfer to these higher level organisms (Lefebvre et al., 2002). The efficiency of transfer is due, in part, to planktivorous fishes being highly mobile. They are able to potentially accumulate toxin from large geographic areas, can ingest high numbers of toxic phytoplankton due to their high filtration efficiency (Durbin and Durbin, 1975; Friedland et al., 1984), and are a direct link from toxic algae to large predators.

In Louisiana, the gulf menhaden (*Brevoortia patronus*) represents a likely vector for the transmission of DA since it shares the same characteristics as the planktivorous fishes mentioned above. As a filter-feeding clupeid fish, gulf menhaden also occupy a similar niche (Ahrenholz, 1991) to the northern anchovy of California food webs. The gulf menhaden is an abundant, estuarine-dependent fish (Lassuy, 1983) that can filter the water column at, potentially, a high rate (as measured in the congener Atlantic menhaden, *Brevoortia tyrannus*, by Durbin and Durbin, 1975). Thus, it may be capable of accumulating large amounts of contaminants. Additionally, gulf menhaden are one of the most abundant fishes in the northern Gulf of Mexico, with Louisiana providing up to 52% of the juvenile abundance for the nation's second largest fishery in terms of landings (Vaughan et al., 2007). They are also identified as a prominent prey item for several upper trophic level predators that are common in Louisiana estuaries, such as brown pelicans, bottlenose dolphins (*Tursiops truncatus*), and several species of shark (Hildebrand, 1963; Snelson et al., 1984; Ahrenholz, 1991; Hoffmayer and Parsons, 2003; Bethea et al., 2004; Barry et al., 2008; US Fish and Wildlife Service, 2008).

One estuary that is at a particularly high risk of DA contamination of the food web in Louisiana is Terrebonne Bay. High amounts of *Pseudo-nitzschia* (10^6 cells l^{-1}) have been reported in this bay and adjacent Gulf of Mexico waters (Dortch et al., 1997), and like most Louisiana estuaries, there is a high abundance of gulf menhaden that reside in and around the Bay. Additionally, many upper trophic level predators (brown pelicans, bottlenose dolphins, sharks, etc.) use this area for foraging and nursery functions (Neer et al., 2007; Barry et al., 2008; US Fish and Wildlife Service, 2008; Miller and Baltz, 2010). Based on previous research and knowledge concerning DA and its vectors, the goal of the present study was to identify whether the gulf menhaden is a potential vector of DA to such higher trophic levels in Terrebonne Bay, Louisiana.

2. Materials and methods

2.1. Water and gulf menhaden sampling and initial processing

Samples were acquired in Terrebonne Bay, Louisiana (29°09′N, 90°38′W) which is a typical representation of a Louisiana estuary with shallow (<2.0 m), generally turbid waters, and a microtidal regime (tidal range <1.0 m). It spans 1761 km^2 and is bordered on the south by three barrier islands with three large connections to the Gulf of Mexico (US Environmental Protection Agency, 1999; Fig. 1). Sampling design was based on Carlson and Brusher (1999) to catch both gulf menhaden and sharks to facilitate the present study and the National Marine Fisheries Service Cooperative Gulf of Mexico States Shark Pupping and Nursery Survey. Specimens were collected during periods when the fishes could be found concurrently in the Bay, monthly from July 2007 to September 2007 and from April 2008 to June 2008 (Fig. 1). Winter collections were not conducted. Gulf menhaden were sampled using a cast net or a 186 m long gillnet that was allowed to soak for 30 min. The monofilament gillnet (donated by the National Marine Fisheries Service) was composed of 6 panels of stretch mesh, ranging from sizes 7.6 to 14.0 cm, in steps of 1.3 cm, while the cast net had a 1.83 m diameter with 0.95 cm mesh. After the net was deployed, GPS location and a suite of environmental parameters were recorded including: temperature, salinity, and dissolved oxygen (recorded *in situ* with a YSI-85 environmental meter); Secchi depth; and water depth (determined using a Raymarine ST40 fathometer). Whole seawater samples were collected from the surface with a 2 l Nalgene bottle. Concentrated plankton samples for species identification were obtained by vertical net tows using a 30 μm mesh size plankton net (Aquatic Research Instruments, Hope, ID, USA). The net tows and subsamples of whole water were preserved using glutaraldehyde solution (2% final concentration) immediately after collection for light microscope observations of *Pseudo-nitzschia* cell abundance and relative community composition. The remaining water samples were brought back to the laboratory at Louisiana Universities Marine Consortium (LUMCON) in coolers and were filtered (25 mm GF/F, Whatman) for toxin measurements (see Section 2.4). After the net was retrieved, gulf menhaden were identified, measured to fork length (FL), and sacrificed in an ice-water bath. Twelve gulf menhaden were chosen at random from each sample set to be used in toxin or stomach contents analyses, and kept in a −20 °C freezer until analysis.

Whole water samples from the nearby Gulf of Mexico adjacent to Terrebonne Bay were also collected for *Pseudo-nitzschia* enumeration and toxin analyses following the same procedure used above. The applicability of these samples as Terrebonne Bay *Pseudo-nitzschia* abundance estimates was investigated using the Wave-Current-Surge Information System for Coastal Louisiana (WAVCIS), with permission from Dr. Gregory Stone at Louisiana State University, to examine the exchange of Bay water with the nearby Gulf of Mexico as indicated by Marmer (1954) and Prager (1992). The water samples were taken from the three stations nearest to Terrebonne Bay, C1 (29°03′N, 90°31′W), C3 (28°59′N, 90°31′W), and C4 (28°57′N, 90°31′W) (Fig. 1), from a monthly sampling effort conducted by researchers at LUMCON. These stations were chosen because they represent the approximate maximum range for the gulf menhaden fishery in coastal Louisiana (Vaughan et al., 2007), and were sampled during the same months as Terrebonne Bay sampling.

2.2. Phytoplankton biomass and plankton community composition

Chlorophyll *a* (Chl *a*) concentrations were determined for samples taken from Terrebonne Bay from all sampling months, except July 2007, to determine the total phytoplankton biomass. Duplicate sample aliquots were filtered through a 25 mm GF/F filter (Whatman), and extracted in 90% acetone (Sigma–Aldrich) at −20 °C for 24 h. Chl *a* concentrations were read on a Turner 10-AU fluorometer in low light to minimize photodegradation (Parsons et al., 1984).