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Crassostrea virginica grazing on toxic and non-toxic diatoms

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ABSTRACT

Despite high abundances of toxic *Pseudo-nitzschia* spp. over Louisiana oyster beds (*Crassostrea virginica*; eastern oyster) there have been no documented cases of amnesic shellfish poisoning (ASP) in the state. Two possible explanations are that oysters do not readily feed on long pointed chains of *Pseudo-nitzschia* cells or they discriminate against toxic cells while grazing. To test these hypotheses, short-term grazing experiments were conducted with several diatoms, including the domoic acid (DA)-producing *Pseudo-nitzschia multiseries* (1.31 ± 0.057 pg DA cell^{-1}) and the non-toxic *Pseudo-nitzschia delicatissima*, *Thalassiosira weissflogii*, and *Ditylum brightwellii*. Grazing rates on the small centric species *T. weissflogii* were significantly higher than on the larger and pointier *D. brightwellii* and either *Pseudo-nitzschia* species. Grazing on toxic *P. multiseries* and non-toxic *P. delicatissima* was not significantly different. Pseudofeces production was higher and feces production was occasionally lower in oysters fed *Pseudo-nitzschia* spp. than in oysters fed the other two diatoms. Our data demonstrate lower filtration rates of *C. virginica* on *Pseudo-nitzschia* spp. relative to the other diatoms tested and comparable filtration on toxic and non-toxic *Pseudo-nitzschia* spp. These findings suggest that eastern oysters do not discriminate amongst food types due to DA content.

1. Introduction

Pseudo-nitzschia is a diatom genus including several species known to produce the neurotoxin domoic acid (DA), a water soluble, heat stable amino acid that causes amnesic shellfish poisoning (ASP) in humans (Perl et al., 1990) and domoic acid poisoning (DAP) in marine mammals and birds (Work et al., 1993b; Hatfield et al., 1995; Leira et al., 1998; Scholin et al., 2000). DA can accumulate in the bodies of primary consumers, such as shellfish (Haya et al., 1991; Mackenzie et al., 1993; Adams et al., 2000; Vale and Sampayo, 2002) and finfish (Lefebvre et al., 2001; Vale and Sampayo, 2001; Costa and Garrido, 2004; Fire and Silver, 2005; Busse et al., 2006), and sicken animals or humans that feed on them (Work et al., 1993b; Scholin et al., 2000). The first reported incident of human illness due to DA exposure via consumption of contaminated shellfish occurred in Prince Edward Island, Canada, in 1987 and brought attention to *Pseudo-nitzschia* as the first toxin-producing diatom (Bates et al., 1989). Characteristic symptoms of ASP and/or DAP include short-term memory loss, dizziness, seizures, confusion, vomiting, diarrhea, uncontrollable scratching, and death (Perl et al., 1990; Work et al., 1993a; Gulland et al., 2002).

Pseudo-nitzschia spp. are widespread in coastal areas of the Gulf of Mexico (Dortch et al., 1997; Pennock and Burns, 2000; Thessen et al., 2005; C. Heil, personal

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communication). In Louisiana coastal waters, total *Pseudo-nitzschia* abundance often exceeds 10^6 cells L^{-1} (Dortch et al., 1997; Thessen et al., 2005), perhaps stimulated by high nutrient loading from the Mississippi River (Parsons et al., 2002). At least seven species have been identified from this region, including four known toxin producers (Thessen et al., 2005), and high levels of DA have been measured in plankton samples (Dortch et al., 2006).

Louisiana leads the United States in the production of eastern oysters (*Crassostrea virginica*), accounting for an average of 34% of the nation's oyster landings from 1997 to 2006. In 2007, the dockside value was about $40 million with a meat yield of 5400 metric tons (LA Department of Wildlife and Fisheries, 2008). In the Gulf of Mexico, oysters are generally harvested from water-bottoms with mean annual salinities between 10 and 20 (Melancon et al., 1998). *Pseudo-nitzschia*, including potentially toxic species, occur and grow in that salinity range (Thessen et al., 2005) and high DA levels have been measured in plankton over oyster beds (Dortch et al., 2006). Despite the proximity of toxic *Pseudo-nitzschia* and oysters, there have been no documented incidences of ASP due to consumption of oysters from the Gulf of Mexico. In order to assess the potential for a future threat of ASP in the Gulf, especially in the light of increasing *Pseudo-nitzschia* abundance in response to eutrophication (Parsons et al., 2002), it is important to explain the current absence of ASP in areas where it might be expected.

One possible explanation for the absence of ASP is that oysters do not readily graze on toxic *Pseudo-nitzschia* (Thessen et al., 2002). Numerous studies show that, in the laboratory, bivalve grazing can be adversely affected by harmful algae (Gainey and Shumway, 1991; Bardouil et al., 1996; Bricelj et al., 2001; Pate et al., 2005; Shumway et al., 2006). Studies focusing specifically on *Crassostrea virginica* grazing on *Pseudo-nitzschia* spp. are surprisingly rare considering the importance and vulnerability of the fishery. Some preliminary work by Roelke (1993) suggested that *C. virginica* can feed on *Pseudo-nitzschia*, but clearance rates were sporadic and independent of cell concentration or toxin content. In recent studies (Mafra et al., 2009a, b) *C. virginica* clearance rates on unialgal suspensions were lower on *P. multiseries* in comparison with the prymnesiophyte *Isochrysis galbana* but independent of *P. multiseries* toxicity, supporting the hypothesis that oysters do not graze readily on *Pseudo-nitzschia*.

To further assess the risk of ASP in areas where harvestable *Crassostrea virginica* are regularly exposed to high *Pseudo-nitzschia* abundance, as would be the case in Louisiana coastal waters, we addressed the following questions. Does DA affect adult *C. virginica* grazing? How does *C. virginica* grazing on *Pseudo-nitzschia* spp. compare with grazing on other diatoms? To answer these questions we performed multiple grazing experiments from 2000 to 2002 using several diatom species of different sizes and toxicities (Table 1). We compared clearance rates of adult oysters fed toxic *P. multiseries* and non-toxic *P. delicatissima*, as well as two other non-toxic diatom species *Thalassiosira weissflogii* and *Ditylum brightwellii*.

Table 1
Summary of details for experiments investigating eastern oyster, *Crassostrea virginica*, grazing on the diatoms *Pseudo-nitzschia multiseries*, *Pseudo-nitzschia delicatissima*, *Thalassiosira weissflogii* and *Ditylum brightwellii*.

Year	Species	*n*	Controls	Duration (min)
2000	*T. weissflogii*	4	1	42
2000	*P. delicatissima*	4	1	78
2000	*D. brightwellii*	4	1	28
2000	*P. delicatissima*	3	1	145
2000	*P. multiseries*	4	1	114
2001	*P. delicatissima*	3	3	307
2001	*P. multiseries*	3	3	209
2001	*P. delicatissima*	3	3	225
2001	*P. multiseries*	3	3	234
2002	*T. weissflogii*	6	3	195
2002	*P. delicatissima*	7	3	301
2002	*D. brightwellii*	7	3	255

2. Materials and methods

2.1. Oyster collection and maintenance

Eastern oysters (*Crassostrea virginica*; 78.1 ± 9.5 mm in height; 8.774 ± 2.578 g wet weight) were collected from Terrebonne and Tambour Bays south of Cocodrie, Louisiana, cleaned with a wire brush and either used immediately (in 2000 and 2001 experiments) or held in an ambient flow-through system for up to 1 week (in 2002 experiments; 8–20 salinity and 20–33 °C). Oysters held in ambient flow-through systems were exposed to natural seston. Accumulated sediment was removed daily by draining the tank and rinsing the oysters and prior to each experiment oysters were again scrubbed.

2.2. Diatom cultures and maintenance

Four species of diatom were used in these experiments, *Pseudo-nitzschia delicatissima* (LaPn-4), *Pseudo-nitzschia multiseries* (MU1), *Thalassiosira weissflogii* (LB 2054) and *Ditylum brightwellii* (NEPCC 8A). LaPn-4 was isolated from coastal Louisiana (Parsons et al., 1999) and MU1 was isolated from Santa Cruz, California (P. Miller; see Thessen et al., 2005). LB 2054 came from UTEX, the culture collection of algae at the University of Texas (Austin, TX, USA). NEPCC 8A was isolated from Patrician Bay, Canada, and obtained from the Canadian Centre for the Culture of Microorganisms at the University of British Columbia (Vancouver, BC, Canada). Cultures were maintained in 55 mL screw capped glass tubes filled with 20–22 mL of medium. The medium was natural seawater (20 salinity) amended with *f*/2 nutrients (Guillard, 1975; Sigma G-9903, Sigma Inc., St. Louis, MO, USA), except that the silicate concentration was doubled and Se was added to a level of 10^{-8} M (Harrison et al., 1988). The *P. delicatissima, T. weissflogii* and *D. brightwellii* cultures were maintained at 24.5 °C with light provided by 34-W cool white fluorescent bulbs (F40CW/RS/SS Sylvania, Panvers, MA, USA) at 50–90 μmol photons m^{-2} s^{-1}. The *P. multiseries* culture was maintained at 19 °C because it did not grow at higher temperatures. All cultures were maintained on a 12:12 h light/dark cycle with lights on at 06:00 h and lights off at