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Effects of the toxic dinoflagellate *Alexandrium monilatum* on survival, grazing and behavioral response of three ecologically important bivalve molluscs

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ABSTRACT

Little is known about interactions between shellfish and the toxic dinoflagellate *Alexandrium monilatum*. Toxic strains produce endotoxins with hemolytic and neurotoxic properties, and have been linked to fish and invertebrate kills. We experimentally assessed the survival, grazing and behavioral responses of three shellfish species to *A. monilatum*. Grazing studies were conducted with two size classes of *Crassostrea virginica*, *Mercenaria mercenaria*, and *Perna viridis*. These species inhabit areas where blooms of *A. monilatum* occur. Clearance rates of each species were depressed when exposed to toxic *A. monilatum* alone or with nontoxic *Pavlova* sp., in comparison to control animals fed only nontoxic algae. Exposure to toxic *A. monilatum* also caused shellfish to decrease shell valve gape. Intact cells of *A. monilatum* were found within shellfish feces, but the cells did not re-establish growing populations following gut passage. Survival of larval *M. mercenaria* and *C. virginica* was also tested when exposed to *A. monilatum* cells. Survival was significantly lower for larvae exposed to sonicated *A. monilatum*, in comparison to control larvae tested with nontoxic *A. tamarense*. Overall, the data indicate that blooms of *A. monilatum* can adversely affect some shellfish species by reducing valve gape and clearance rate, and by inducing larval mortality.

1. Introduction

Outbreaks of harmful algal species have increased in frequency, intensity and geographic distribution, causing public health and economic impacts (Hallegraeff, 1993; Ramsdell et al., 2005). Harmful algae include species that produce toxins or otherwise harm organisms directly or indirectly (see reviews in Shumway, 1990; Burkholder, 1998). "Toxic algae" is a term used in reference to species that produce toxic strains. Such species typically show a range in toxicity including some strains that are apparently unable to express toxicity (see reviews in Burkholder et al., 2005; Burkholder and Glibert, 2006). Nutrients added to coastal regions from cultural eutrophication are one noted cause of increased blooms (Anderson et al., 2002; Glibert et al., 2005), and the movement of shellfish stocks to different areas is one mechanism that may introduce some species of harmful dinoflagellates to new areas, as some are able to pass intact and viable through the shellfish digestive tract (Shumway et al., 1985b; Bricelj et al., 1993; Laabir and Gentien, 1999; Bauder and Cembella, 2000; Springer et al., 2002; Laabir et al., 2007; Hégaret et al., 2008a,b).

Dinoflagellate toxins can contaminate seafood and cause human illness and death when filter-feeding bivalves accumulate and concentrate these toxins that subsequently can be transferred up the food chain (Shumway, 1990; Burkholder, 1998; Landsberg, 2002). Toxin accumulation by shellfish is influenced by grazing rates and behavioral responses. Historically, shellfish were regarded as unaffected vectors of algal toxins, but effects of toxic algae on shellfish behavior, metabolism and survival have increasingly been recognized (Shumway and Cucci, 1987; Shumway and Gainey, 1992). Bivalve molluscs may close shell valves or reduce filtration rate when exposed to toxic dinoflagellates, which decreases exposure to the bloom (e.g. Shumway et al., 1985a; Shumway and Cucci, 1987; Gainey and Shumway, 1988a; Lesser and Shumway, 1993; Lassus et al., 1999; Hégaret et al., 2007). Toxins produced by dinoflagellates have caused shellfish mortalities, and toxic dinoflagellates can also affect shellfish recruitment and survival by causing

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behavioral alterations, depressed feeding, and impaired reproduction and growth (see reviews by Shumway, 1990; Burkholder, 1998; Landsberg, 2002).

The potentially toxic, chain-forming dinoflagellate *Alexandrium monilatum* (Howell) Balech blooms along the east coast of Florida (Howell, 1953; Norris, 1983), north to Chesapeake Bay (Morse, 1947), the Gulf of Mexico (Mississippi Sound, Perry et al., 1979; Texas, Connell and Cross, 1950; Gunter, 1942; Ray and Aldrich, 1967), South America and Central America (Venezuela, Costa Rica, Caribbean Sea, Halim, 1967; Ferraz-Reyes et al., 1985) and the Pacific Ocean off Ecuador (Balech, 1995) (Table 1). Blooms of *A. monilatum* have been associated with mortalities of fish and invertebrates (Table 1). Recently, an *A. monilatum* bloom in the lower York River, Virginia, USA caused mortalities of veined rapa whelks (*Rapana venosa*; Harding et al., 2009). Connell and Cross (1950) described a bloom of *A. monilatum* in Offats Bayou near Galveston, TX, USA, during the summer 1949, and linked sewage pollution to stimulation of the bloom. This bloom occurred immediately after heavy precipitation flushed sewage-polluted surface water into the bayou. Also, areas in which heavy growth of this organism recurred frequently were in wind-sheltered areas of the bayou where effluents from private septic tanks infiltrated the estuary. Based upon the minimum nitrogen cell quota of this species, it has been suggested that high N flux would be required to support development of blooms of *A. monilatum* (Juhl, 2005).

The toxin(s) from *A. monilatum* cause paralysis and mortality in finfish (Gates and Wilson, 1960) and have been shown to be toxic to homeotherms (Erker et al., 1982; Erker et al., 1985). Heating or freezing *A. monilatum* cells increased fish mortality in laboratory experiments, suggesting that this dinoflagellate produces endotoxins released with cell lysis, and maximal toxicity was reported in senescent cultures with high cell autolysis (Aldrich et al., 1967). Ray and Aldrich (1967) found that oysters (*Crassostrea virginica* Gmelin) rarely opened shell valves or filtered when exposed to *A. monilatum*. Polychaetes (*Polydora* sp.) inhabiting the oyster shells, along with fish in separate bioassays, had high mortality rates. Sievers (1969) compared the toxicity of a strain of *A. monilatum* versus a strain of *Karenia brevis* to annelids, crustaceans, molluscs and finfish (sheepshead minnow, *Cyprinodon variegatus* Lacepede). Sheepshead minnows were sensitive to both dinoflagellate species, but mean times to death indicated that they were more sensitive to the *K. brevis* strain than to *A. monilatum*. In contrast, the annelids and molluscs were more sensitive to *A. monilatum* than to *K. brevis*, and the crustaceans were resistant to both species of dinoflagellate tested.

Schmidt and Loeblich (1979) found paralytic shellfish toxins (saxitoxin and gonyautoxins) in laboratory cultures of *A. monilatum*. An extract of *A. monilatum* had neurotoxic and hemolytic properties and was chemically different from saxitoxin and related compounds (Clemons et al., 1980; Bass et al., 1983; Erker et al., 1985). A toxin produced by *A. monilatum* was purified and identified as goniodomin A (Hsia et al., 2005), which also is produced by *Alexandrium pseudogoniaulax* (Murakami et al., 1988). The purified toxin exhibited hemolytic activity (P. Moeller, National Oceanic and Atmospheric Administration – National Ocean Service [NOAA-NOS], Charleston, SC, USA, personal communication, January 2008). Extracts of *A. monilatum* cells were hemolytic to erythrocytes from several mammalian species including humans, and lethal to cockroaches, guppies and mice (Clemons et al., 1980; Bass et al., 1983).

The shellfish species selected for this research are important ecologically and commercially along the East and Gulf Coasts of the U.S. and inhabit areas where blooms of *A. monilatum* have occurred. The objectives of this study were to: (1) examine the effects of *A. monilatum* at bloom density upon clearance rates of eastern oysters (*C. virginica*), northern quahogs (*M. mercenaria* Linnaeus), and green mussels (*P. viridis* Linnaeus); (2) evaluate shellfish behavior (valve gape) in response to *A. monilatum*; (3) assess impacts of *A. monilatum* upon the survival of larval *C. virginica* and *M. mercenaria*; and (4) assess survival of *A. monilatum* after ingestion by shellfish to gain insights into whether or not shellfish may act as vectors for the introduction of *A. monilatum* if transported to new areas.

Table 1
Historic record of blooms of *Alexandrium monilatum* in southeastern and Gulf Coast ecosystems (n.a. = not available; also see Landsberg, 2002; Juhl, 2005).

Location	Date	Reported mortality	Density (Cells L^{-1})	Salinity	Temperature (°C)	Reference
Indian River and Sarasota, FL	August–September 1951	Finfish	n.a.	18–32	30–34	Howell (1953)
Offats Bayou, Galveston, TX	Summer 1949	Finfish, shrimp, crabs	n.a.	n.a.	n.a.	Connell and Cross (1950)
Fort Myers to Naples, FL	1966	Finfish – *Caranx* spp., *Strongylura marina*, *Lagodon rhomboids*	n.a.	>32	>29	Williams and Ingle (1972)
Galveston, TX[a]	1971–1972	Finfish, shellfish, annelids, coelenterates, crustaceans, echinoderms	1.88×10^6 "massive bloom"	30–34	29–32	Wardle et al. (1975)
Indian River, FL	July 1977	n.a.	8.9×10^5	32.0	31.5–32	Norris (1983)
Melbourne Beach, FL	July 1977	n.a.	1.7×10^6	30.5	29.5	Norris (1983)
Port St. John, FL	September 1977	Finfish (thousands)	Bloom	n.a.	n.a.	Norris (1983)
Mississippi Sound (MS-LA)	August 1979	No deaths	1.65×10^7	24–26	30–31	Perry et al. (1979)
Mobile Bay, AL	August 1979	Finfish	n.a.	n.a.	n.a.	Perry et al. (1979)
Pensacola Bay, FL	August 1979	Finfish	3.18×10^7	14	28	Perry et al. (1979)
Indian River, FL	September 1979	n.a.	$<1 \times 10^3$	16–21	24.5–30	Norris (1983)
Gulf of Cariaco, Venezuela	January–February, April–May 1984; January 1985	n.a.	0.1 to 4×10^4	n.a.	n.a.	Ferraz-Reyes et al. (1985)
Coastal MS	1998	Zooplankton, ichthyoplankton	n.a.	n.a.	n.a.	ICES (1999)
York River, VA	September 2007	Veined rapa whelks (*Rapana venosa*)	4.0×10^7	22.4–22.8	27–28	Harding et al. (2009)

[a] 30 species: *Americonuphis magna*, *Anadara brasiliana*, *Anadara ovalis*, *Arenaeus cribarius*, *Bascanichthys scuticaris*, *Bunodosoma cavernata*, *Callinectes sapidus*, *Callinectes similis*, *Clibanarius vittatus*, *Crassostrea virginica*, *Cyprinidon variegates*, *Donax variabilis*, *Emerita benedicti*, *Gobiesox punctulatus*, *Hepatus epheliticus*, *holothuroids*, *Hypleurochilus geminatus*, *Isocheles wurdemanni*, *Mellita quinquesperforata*, *Menippe mercenaria*, *Micropholis atra*, *Nereis* sp., *Oliva sayana*, *Petrolisthes armatus*, *Polinices duplicata*, *Porcellana sayana*, *Spisula solidissima*, *Siphonaria pectinata*, *Terebra cinerea*, *Thais haemastoma*.