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Harmful algal toxins of the Florida red tide (*Karenia brevis*): natural chemical stressors in South Florida coastal ecosystems

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Abstract

The Florida red tide is a descriptive name for high concentrations of the harmful marine alga, *Karenia brevis*. Although most prevalent along the south-west Florida coast, periodic blooms have occurred throughout the entire US and Mexico Gulf coasts and the Atlantic coast to North Carolina. This dinoflagellate produces a suite of polyether neurotoxins, called brevetoxins, that cause severe impacts to natural resources, as well as public health. These naturally produced biotoxins may represent one of the most common chemical stressors impacting South Florida coastal and marine ecosystems. Impacts include massive fish kills, marine mammal, sea turtle and sea bird mortalities, benthic community die-off and public health effects from shellfish contamination and inhalation of air-borne toxins. The primary mode of action is binding to voltage-gated sodium channels causing depolarization of nerve cells, thus interfering with nerve transmission. Other effects include immune depression, bronchial constriction and haemolysis. Parent algal toxins are synthesized within the unicellular organism, others are produced as metabolic products. Recent studies into the composition of brevetoxins in cells, water, air and organisms have shown PbTx-2 to be the primary intracellular brevetoxin that is converted over time to PbTx-3 when the cells are ruptured, releasing extracellular brevetoxins into the environment. Brevetoxins become aerosolized by bubble-mediated transport of extracellular toxins, the composition of which varies depending on the composition in the source water. Bivalved molluscs rapidly accumulate brevetoxins as they filter feed on *K. brevis* cells. However, the parent algal toxins are rapidly metabolized to other compounds, some of which are responsible for neurotoxic shellfish poisoning (NSP). These results provide new insight into the distribution, persistence and impacts of red tide toxins to south-west Florida ecosystems.

Keywords

Brevetoxins; Biotoxins; PbTx; HABs; Florida red tide

Introduction

Toxic red tides (harmful algal blooms, HABs) were reported along the Florida Gulf coast by Spanish explorers in the 1500s with written documentation in the 1840s (Ingersoll 1882; Walker 1884; Baden et al. 2005). Environmental impacts include massive fish kills, marine mammal, sea turtle and sea bird mortalities and impacts on benthic communities including sea grass and coral community die-offs (Steidinger et al. 1973, 1995; Bossart et al. 1998; Trainer and Baden 1999; Flewelling et al. 2005). Human health effects include cases of human intoxication from neurotoxic shellfish poisoning (NSP) and impacts on human respiratory function (Plakas et al. 2002; Fleming et al. 2005; Pierce et al. 2005).

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Globally, HABs appear to be increasing in frequency, duration and geographic region (Smayda 1990; Anderson 1994; Van Dolah 2000). The most prevalent HABs along the Florida Gulf coast are caused by the dinoflagellate, *K. brevis* (formerly, *Gymnodinium breve*, Davis) (Duagbjerg et al. 2001). Although always present in low concentrations throughout the Gulf with no apparent adverse effects, high concentrations (blooms) of *K. brevis* produce sufficient neurotoxins to cause the ecological and human impacts (Roberts 1979; Tester and Steidinger 1997; Landsberg and Steidinger 1998; Poli et al. 2000; Pierce et al. 2004, 2005). Commonly referred to as the Florida Red Tide, these blooms occur most frequently along the south-west Florida coast that vary from minor blooms to severe, intensive blooms that persist up to 18 months (Gunter et al. 1948; Rounsefell and Nelson 1966; Landsberg 2002).

Brevetoxins are polycyclic ether ladder compounds that are differentiated into two similar, yet distinct backbone structures PbTx-1 (type-A) with 10 rings, and PbTx-2 (type-B) with 11 rings (Fig. 1) (Catterell and Risk 1981;Poli et al. 1986;Shimizu et al. 1986). The suite of brevetoxins includes at least nine, and as many as 14 compounds (designated as PbTx-1, -2, -3, etc.) ranging in molecular weight from 868 to 936 (Poli et al. 1986;Baden et al. 1995). The parent algal toxins (PbTx-1 and -2) undergo metabolic changes producing the suite of PbTx as the bloom matures. Several additional PbTx compounds and analogue have been observed recently and are currently undergoing structural verification, including the PbTx antagonist, brevenal, that competitively inhibits the toxicity of PbTx (Baden et al. 2005;Bourdelais et al. 2005;Abraham et al. 2006).

The primary mode of action is binding of PbTx to site 5 of voltage-sensitive sodium channels (VSSC) in neurons (Baden and Mende 1982; Trainer and Baden 1999). This binding results in persistent activation of cells, interfering with proper nerve transmission. Additional impacts include bronchial constriction, haemolysis, immune suppression and genetic damage (Baden et al. 1995, 2005; Bossart et al. 1998). Mammalian toxicity has been determined by oral, intravenous (iv) and intrapperitoneal (ip) injections to mice. PbTx-3 (T17) exhibited an acute 24 h LC_{50} of 0.52 mg/kg oral, 0.17 mg/kg ip and 0.09 mg/kg iv. PbTx-2 (T34) exhibited 6.6 mg/kg oral, 0.2 mg/kg iv and 0.2 mg/kg ip, indicating PbTx-3 to be the predominantly orally toxic PbTx (Baden and Mende 1982). The 24 h acute LD_{50} for PbTx-1 has been established as 0.5 mg/kg ip, mouse body weight (Rein et al. 1994).

The most serious intoxication observed in humans is NSP resulting from ingestion of contaminated shellfish (Steidinger et al. 1973; Baden 1983; Poli et al. 2000). Filter-feeding shellfish, such as mussels, clams and oysters, accumulate the neurotoxins and toxic metabolites as they feed on the microscopic algae. Symptoms of NSP include gastrointestinal disorders, tingling sensation of lips and extremities, reversal of hot and cold sensation, rapid heartbeat, loss of balance and a lack of motor coordination (Steidinger and Baden 1984; Baden et al. 1995). The State of Florida maintains a public health safety policy for closure of shellfish beds at *K. brevis* cell counts of 5,000 cells/l, requiring continued monitoring of shellfish waters for public health protection. Human toxicity levels have been established as 0.8 mg PbTx-2/kg shellfish tissue. Utilizing the mouse bioassay as the standard technique for monitoring shellfish beds, toxicity is indicated at 20 mouse units/100 g of shellfish tissue (American Public Health Association 1970). New analytical techniques are currently being developed to provide more rapid and less invasive procedures, including enzyme-linked immunosorbent assay (ELISA) (Naar et al. 2002).

Another route of exposure is inhalation of air-borne (aerosol) PbTx. The aerosolization process occurs by bubble-mediated transport of PbTx molecules adsorbed to the surface of entrained bubbles. Bubbles transport toxins to the sea surface where they are ejected into the air associated with jet and film drops produced as the bubbles burst (Blanchard 1975; Pierce et al. 1990). Toxin-containing aerosols are blown onshore causing respiratory irritation to humans

and other mammals (Cheng et al. 2005; Pierce et al. 2005; Baden et al. 2005). Symptoms of exposure include stinging eyes and nose, dry choking cough and onset of asthmatic attack in susceptible subjects (Fleming et al. 2005; Kirkpatrick et al. 2006). The first written report associating respiratory irritation with a Florida red tide was provided by Taylor (1917). A more thorough correlation with symptoms was provided by Woodcock (1948) who surmised that the irritation was caused by aerosolized products of the dinoflagellate bloom. More recent studies have verified Woodcock's hypothesis showing the composition of aerosolized PbTx and the size distribution of toxin-containing aerosols (Pierce et al. 1990, 2003, 2005; Cheng et al. 2005).

Massive fish kills are a common result of the Florida red tide leaving beaches and bays choked with dead and decaying carcasses. The decomposing biomass adds to the environmental stress by depleting oxygen from the water. Fish accumulate PbTx by ingestion of viable *K. brevis* cells and contaminated prey, and by absorption of toxins across gill membranes (Abbott et al. 1975; Catterell and Risk 1981; Tester et al. 2000). Quick and Henderson (1974) reported a value of 2.5×10^6 cells/l as the typical concentration causing fish mortality. Mortality from chronic exposure to much lower concentrations also has been reported indicating accumulation of toxins to a lethal level over several days (Landsberg 2002; Pierce unpublished data). Fish have the ability to detoxify PbTx and will recover if removed from red tide contaminated water in time. Mortality of benthic invertebrates has been related to anoxia as well as neurotoxicity resulting from a combination of oxygen depletion by decomposition of dead flora and fauna and night time respiration of high concentrations of *K. breve* (Simon and Dauer 1972; Steidinger et al. 1973; Roberts et al. 1979).

Exposure of marine animals to PbTx results from ingestion of contaminated food and from inhalation of aerosolized PbTx. During two of the largest red tide events to on record, 1946–1947 and 1953–1955, mass mortalities of marine animals were reported all along the central and south-west Florida Gulf coast from Tarpon Springs to Key West (Steidinger et al. 1973; Landsberg 2002). In 1987–1988, more than 740 bottlenose dolphins died along the Atlantic coast, implicating PbTx-contaminated menhaden as the vector (Geraci 1989). Ingestion of contaminated fish prey was implicated as the cause in the deaths of 107 bottlenose dolphins along the Florida Panhandle in 2004 (Flewelling et al. 2005). Manatee deaths have been reported during numerous red tide events, including 39 deaths in 1982, 149 in 1996, 34 in 2002 and 86 in 2005 (O'Shea et al. 1991; Bossart et al. 1998; FWCC 2007). PbTx and metabolites were found to persist in seagrass communities long after the bloom had gone, providing a source for continued intoxication of grazing manatees (Flewelling et al. 2005).

As a result of the extensive areas and variety of natural resources impacted by *K. brevis* blooms, PbTx produced by the Florida red tide may represent the most common suite of chemical stressors impacting South Florida coastal and marine ecosystems. The purpose of this paper is to provide a comprehensive assessment of PbTx produced during Florida red tides, considering chemical composition, transport, exposure, concentration and toxicity.

Methods

PbTx composition

The composition and concentration of PbTx were investigated from red tide blooms along the south-west Florida Gulf coast providing representative results for blooms from Tampa Bay to the Florida Keys. Water samples were collected in 1 l glass bottles near-shore, and aboard ship using Niskin® bottles attached to a rosette sampler. A 20 ml sub-sample was fixed with Utermohls solution (Guillard 1973) for microscopic identification and enumeration of *K. brevis* cells. PbTx were extracted from the water by filtration through a C-18 solid-phase

extraction disc (Ansys Technologies Inc., Lake Forest, CA, USA) and recovered with methanol for LC–MS analyses according to the procedure of Pierce et al. (2005).

PbTx analyses were performed by LC–MS using a ThermoFinnigan AqA HPLC/MS obtained from Thermo Electron Corp. (Manchester, UK) using an AqA single quad system scanned from 204 to 1,216 AMU with a Phenomenex Luna C-18 5Fm 250 mm × 2 mm analytical column with solvent gradient. The instrument was calibrated with a standard PbTx mix containing PbTx-2 and PbTx-3 obtained from the Center for Marine Science, UNCW, Wilmington, NC (Pierce et al. 2005).

The composition of intra and extracellular toxins was studied using a low pressure cell concentrator (Amicon, Beverly, MA) to separate viable cells from ambient water. The unarmored nature of *K. brevis* renders it susceptable to cell lysis releasing PbTx to the ambient water. Extracellular toxins were recovered from the filtrate by C-18 disc as above. Intracellular toxins were recovered by lysing the *K. brevis* cells with distilled water and filtering through a C-18 disc, followed by methanol extraction for LC–MS analysis as above. One-litre samples were analysed for total toxins, for comparative mass balance (Pierce et al. 2001).

Aerosol toxins

Marine aerosol samples were collected along Florida beaches with high-volume air samplers (TE-5000, Tisch Environmental Inc., Village of Cleaves, OH, USA) fitted with 20 cm × 28 cm glass fibre filters (Whatman EPM 2000, Maidstone, England) (Pierce et al. 2003, 2005). Toxin-containing aerosol particle size was determined with a 5-stage, high-volume cascade impactor, according to the procedures of Cheng et al. (2005). A portable, solar powered weather station also was deployed to provide wind speed and direction, temperature and relative humidity (Complete Weather Station, Davis Instruments, Hayward, CA, USA). PbTx in marine aerosol were recovered from the glassfibre filters by extraction for 12 h in acetone using a Soxhlet apparatus and transferred to methanol for LC–MS analysis as above (Pierce et al. 2005).

Shellfish toxins

Clams (*Merceneria merceneria-campenchenesis*) and oysters (*Crassostrea virginica*) were collected from a common site in Sarasota Bay, Florida, before, during and after *K. brevis* blooms. Four-litre water samples were collected concurrently with shellfish for *K. brevis* cell counts and PbTx analyses. PbTx and metabolites were extracted from 1 g aliquots of shellfish tissue in acetone with additional separation and clean up by solid-phase extraction in preparation for LC/MS analysis at the US Food and Drug Administration Gulf Coast Seafood Lab, Dauphin Isl., AL (Plakas et al. 2002). Toxicity of the shellfish tissue was determined by mouse bioassay by injecting diethyl ether extracts of 100 g samples of homogenized shellfish meat into mice (American Public Health Association 1970). This assay was performed by the Florida Fish and Wildlife Research Institute, St. Petersburg, FL, reported as mouse units/100 g (Pierce et al. 2004). LC–MS analysis on shellfish tissue was performed using Agilent Technologies (Palo Alto, CA) Model 1100 LC system and Thermo Finnigan (San Jose, CA) Navigator AQA MS detector with electrospray ion source, on a YMC J'Sphere ODS-L80 S-4, 2.0 × 250 mm column (YMC Inc., Wilmington, NC) under gradient conditions (Plakas et al. 2002). MS Standards of pure PbTx-2 and PbTx-3 were provided by the Center for Marine Science, UNC, Wilmington.

Results

PbTx composition

Results of PbTx composition in selected water samples collected along the south-west Florida coast are given in Table 1, along with corresponding *K. brevis* cell counts. In all instances, the total brevetoxin concentrations were sufficient to cause localized fish kills, NSP to exposed filter-feeding molluscs and respiratory irritation from aerosolized brevetoxins. These data provide a representation of the composition, concentration and distribution of PbTx that impact the south-west Florida coast during red tide events. Results of intra and extracellular toxin studies are shown in Table 2 for PbTx analyses in water samples collected in Sarasota Bay during a *K. brevis* bloom from 6 July to 23 November, 2005. These results show the conversion of PbTx-2 to PbTx-3 after rupture (lysing) of *K. brevis* cells, and the persistence of PbTx-3 in the water after the cell count has diminished to below detectable levels.

Aerosol toxins

Results of PbTx monitored in surf water and marine aerosol along a Florida beach during an on-shore red tide bloom (Table 3) show changes in relative concentrations of the major brevetoxin compounds during the transition from water to aerosol (Pierce et al. 2005).

Shellfish (NSP) toxins

Results of LC–MS analysis of NSP-contaminated shellfish (clams and oysters) are given along with the toxicity as mouse units (Table 4). Also provided are the *K. brevis* cell counts and PbTx composition in ambient water samples to provide the composition of the PbTx source. The LC–MS analyses of shellfish tissue were provided to identify the chemical components primarily responsible for NSP toxicity.

Discussion

PbTx composition

The three most abundant PbTx in south-west Florida red tide blooms (Table 1) were the parent algal toxins, PbTx-1 and PbTx-2, and PbTx-3, the primary reduction product of PbTx-2. Also present was the antagonist, brevenal (Bourdelais et al. 2005). The presence of brevenal is significant because it inhibits the toxicity of PbTx by competitively replacing brevetoxins at the sodium channel-binding site. The concentration of brevenal required to inhibit toxicity in fish in the natural environment has not yet been established. The high variability observed for each toxin and for *K. brevis* cell counts is a reflection of the variability of red tide phytoplankton blooms. The amount of *K. brevis* cells observed were representative of medium to high bloom conditions as defined by the Florida Fish and Wildlife Research Institute: background 0–1,000; very low 1,000 < 10,000; low 10,000 < 100,000; medium 100,000 < 1,000,000; high > 1,000,000 cells/l. Therefore, the concentration of PbTx was sufficient to cause fish kills and respiratory irritation.

Analysis of intracellular and extracellular PbTx (Table 2) provides insight into the most abundant toxins affecting marine animals according to the route of exposure. These data show the three most abundant toxins to be PbTx-1, -2 and -3. Most notable is that PbTx-2 was the primary intracellular PbTx. As toxins were released from the cells, the composition changed such that the most abundant extracellular toxin was PbTx-3. Extracellular toxins remained in the water column after detectable levels of *K. brevis* had gone (Table 2) suggesting the need for monitoring PbTx concentration as well as *K. brevis* cell counts for natural resource and public health protection.

The average amount of PbTx produced per cell has been observed to be between 8 and 30 pg/cell (Baden and Tomas 1988; Pierce et al. 2001). Natural background concentrations of 1,000 cells/l or less result in potential PbTx concentrations from 0 to 30 ng/l with no observable adverse effects. However, a slight increase to 5,000 cells/l (about 100 ng/l PbTx) is sufficient to cause filter-feeding shellfish to become toxic for human consumption.

Ichthyotoxicity varies according to the route and rate of exposure and the size and species of fish. The most ichthyotoxic PbTx is PbTx-1 with a 24-h LD-50 of 3.1 nM (2.7 μg/l), followed by PbTx-2 at 14.3 nM (12.8 μg/l) and PbTx-3 at 15.8 nM (14.2 μg/l) (Baden 1983; Rein et al. 1994). Subacute exposure of PbTx to the Japanese rice fish, medaka (*Oryzias latipes*), was found to produce developmental toxicity in embryos at LD-50 of 4 ng/egg (Coleman and Ramsdell 2003). These concentrations were well within environmentally relevant amounts to which fish and fish eggs are exposed, indicating the potential for acute as well as subacute effects resulting from *K. brevis* blooms.

Aerosol toxins

Results of PbTx-containing aerosol (Table 3) showed PbTx-3 as the most abundant with less PbTx-2 and no detectable PbTx-1. A comparison with the source water shows that the most abundant toxin in water was PbTx-2, followed by PbTx-3 and low concentrations of PbTx-1. The antagonist, brevenal, was present in water with trace amounts detected in aerosol (Cheng et al. 2005) indicating possible inhibition of aerosolized PbTx toxicity (Abraham et al. 2005). It is important to note that on 3/30/03, as the *K. brevis* cell count and PbTx concentration increased in the water, the amount of PbTx in marine aerosol dropped drastically. This was a result of a shift in wind direction from on-shore to along-shore showing the importance of wind direction and velocity for respiratory impact (Pierce et al. 2005;Cheng et al. 2005). Particle size distribution analyses of PbTx-containing marine aerosol provided insight into the actual dosages to which study subjects were exposed: upper airway (75–84%) and the lower airways (2–6%) (Cheng et al. 2005). Most significant was the observation that subjects with asthma experienced both upper and lower airway symptoms as well as changes in pulmonary function, even when exposed to low to moderate levels of aerosolized PbTx while healthy subjects did not exhibit reduced pulmonary function (Fleming et al. 2005;Backer et al. 2005).

Studies of bronchoconstriction caused by inhalation of PbTx-2 and PbTx-3 in the guinea pig, showed PbTx-3 to be the agent primarily responsible for respiratory irritation during episodes of Florida red tide (Baden et al. 1982). Increased sensitivity of asthmatics to inhaled PbTx was confirmed by exposing asthmatic sheep to environmentally relevant concentrations of PbTx-3 (Abraham et al. 2005). The seriousness of public health-related effects from red tides was brought to light through a review of emergency room admittance records that revealed a 54% increase in cases of respiratory-related hospital emergency room admissions of coastal residents during an intensive red tide period as compared to similar periods with no red tide activity (Kirkpatrick et al. 2006).

Shellfish (NSP) toxins

Analysis of NSP-contaminated shellfish tissue from Sarasota Bay showed that the parent algal toxins (PbTx-1, -2, -3, etc., defined here as brevetoxins produced in *K. brevis* cells) were not detected in shellfish tissue, except for one oyster sample that contained 0.38 μg PbTx-3/g tissue. The primary compounds observed in shellfish were PbTx-conjugates, identified by LC–MS (*m/z* 1,018, 1,034, 990 and 1,006) produced by metabolic action of shellfish on the algal toxins. These compounds included the cysteine-PbTx-2 conjugate (*m/z* 1,018) and its sulfoxide product (*m/z* 1,034), along with cysteine-PbTx-1 conjugate (*m/z* 990) and its sulfoxide product (*m/z* 1,006) (Poli et al. 2000; Plakas et al. 2002; Wang et al. 2004).

These results (Table 4), show that the oysters exhibited rapid accumulation and reduction in the concentration of metabolites, whereas clams exhibited slower rate of accumulation with longer persistence. Also, oysters exhibited a more rapid onset of toxicity than clams, whereas clams retained their toxicity longer than oysters after the red tide cells diminished to background levels. The more rapid rate of accumulation and toxicity in oysters reflects their higher filtration rate. These results suggest that the two species may be producing different concentrations (and possibly different compounds) of NSP-toxic components. Results indicate that shellfish exhibited toxic levels of NSP compounds once the *K. brevis* cell counts exceeded 5,000 cells/l. Although clams and oysters were toxic by mouse bioassay, little or no parent PbTx was detected in the tissue, implicating PbTx-metabolites as likely agents contributing to NSP toxicity (Plakas et al. 2004;Wang et al. 2004). It is important to note that PbTx-neurotoxins persisted in the water after *K. brevis* cell counts dropped to undetectable (background) levels and that shellfish remained toxic, as observed by mouse bioassay (>20 MU/100 g tissue), for about 4 weeks following the bloom, indicating a persistent risk to public health as well as to the marine ecosystem (Pierce et al. 2004).

Ecosystem effects

The Florida red tide organism, *K. brevis*, is part of the natural phytoplankton community along the south-west Florida coast. However, recent intensive and frequent blooms have raised speculation that human activities are generating excessive nutrient runoff resulting in bloom conditions that exceed the natural balance. Whatever the cause, the south-west Florida coastal ecosystem has, for years, experienced episodes of severe stress from *K. brevis* red tide blooms. This stress is manifest as massive fish kills, marine mammal, sea turtle and sea bird mortalities, invertebrate mortalities and severe respiratory effects.

Although acute effects from red tides are obvious, little is known about long-term exposure to sublethal concentrations such as survival and development of vertebrate and invertebrate larvae (Leverone et al. 2006). Trophic transfer and accumulation of PbTx and toxic analogues produces toxic stress at various trophic levels, also providing reservoirs of neurotoxins for continuous, long-term exposure of marine animals and humans (Tester et al. 2000; Flewelling et al. 2005).

In addition to direct impacts from red tide neurotoxins, oxygen depletion creates stress especially on benthic organisms such as sea grass and patch reef communities, requiring years for recovery (Simon and Dauer 1972; Steidinger et al. 1973). Other indirect stresses include pathogenic bacteria that have been documented in HABs (Buck and Pierce 1989; Epstein 1995; Landsberg 1997; Douchette et al. 1998). PbTx suppression of mammalian immune function has been implicated in mass mortalities of both dolphins and manatees (Geraci 1989; Bossart et al. 1998; Sayer et al. 2005). These hypotheses have been supported by a significant reduction in proliferation of manatee lymphocytes in cell culture exposed to 400 ng/ml PbTx (Walsh et al. 2005).

The extensive evidence for frequent and severe impacts, both acute and subacute, of PbTx and PbTx-analogues on marine life and public health along the Florida Gulf coast points to the need for recognition of these biotoxins as significant chemical stressors within south-west Florida coastal ecosystems. To address these concerns, an ecosystem-based approach is needed to investigate the acute and subacute impacts resulting from *K. brevis* blooms.

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References

Abbott, BC.; Siger, A.; Speigelstein, M. Toxins from the blooms of *Gymnodinium breve*. In: LoCicero, VR., editor. Proceedings of the first international conference on toxic dinoflagellate blooms. Science and Technology Foundation; Wakefield, MA: 1975. p. 355-366.

Abraham WM, Bourdelais AJ, Ahmed A, Serebriakov I, Baden DG. Effects of inhaled PbTx in allergic airways: toxin–allergen interactions and pharmacologic intervention. Environ Health Perspect 2005;113:632–637. [PubMed: 15866776]

Abraham A, Plakas SM, Wang Z, Jester ELE, El Said KR, Granade HR, et al. Characterization of polar brevetoxin derivatives isolated from *Karenia brevis* cultures and natural blooms. Toxicon 2006;48:104–115.10.1016/j.toxicon.2006.04.015 [PubMed: 16808941]

American Public Health Association. Subcommittee on laboratory methods for the examination of shellfish. Vol. 4. American Public Health Association; Washington, DC: 1970. Recommended procedures for the examination of sea water and shellfish; p. 61-66.

Anderson DM. Red tides. Sci Am 1994;271:62–68. [PubMed: 8066432]

Backer LC, Kirkpatrick B, Fleming LE, Cheng YS, Pierce R, Bean JA, et al. Occupational exposure to aerosolized PbTx during Florida red tide events: impacts on a healthy worker population. Environ Health Perspect 2005;113:644–649. [PubMed: 15866778]

Baden DG. Marine food-borne dinoflagellate toxins. Int Rev Cytol 1983;82:99–150.10.1016/S0074-7696(08)60824-4 [PubMed: 6352551]

Baden DG, Mende TJ. Toxicity of two toxins isolated from *Ptychodiscus brevis*. Toxicon 1982;20:457–461.10.1016/0041-0101(82)90009-5 [PubMed: 6896247]

Baden, DG.; Tomas, CR. Variations in major toxins composition for six clones of *Ptychodiscus brevis*. In: Okaichi, T.; Anderson, DM.; Nemoto, T., editors. Red tides: biology, environmental science and toxicology. Elsevier; New York, NY: 1988. p. 415-418.

Baden DG, Mende TJ, Bikhazi G. Bronchoconstriction caused by Florida red tide toxins. Toxicon 1982;20:929–932.10.1016/0041-0101(82)90081-2 [PubMed: 6891120]

Baden, DG.; Flemming, LE.; Bean, JA. Marine toxins. In: DeWolf, FA., editor. Handbook of clinical neurology. Vol. 21. Elsevier; New York: 1995. p. 1-34.

Baden DG, Bourdelais AJ, Jacocks H, Michelliza S, Naar J. Natural and derivative PbTx: historical background, multiplicity, and effects. Environ Health Perspect 2005;113:621–625. [PubMed: 15866774]

Blanchard, C. Bubble scavenging and the water-to-air transfer of organic matter in the sea. In: Baier, R., editor. Applied chemistry at protein interfaces, Advance in Chemistry Series. American Chemical Society; Washington, DC: 1975. p. 145-360.

Bossart GD, Baden DG, Ewing RY, Roberts B, Wright S. Brevetoxicosis in Manatees (*Trichechus manatus latirostris*) from the 1996 epizootic: gross, histologic, and immunohistochemical features. Toxicol Pathol 1998;26:276–282. [PubMed: 9547868]

Bourdelais J, Jacobs HM, Wright JLC, Bigwarfe PM, Baden DG. A new polyether ladder compound produced by the dinoflagellate, *Karenia brevis*. J Nat Prod 2005;68:2–6.10.1021/np049797o [PubMed: 15679307]

Buck JD, Pierce RH. Bacteriological aspects of Florida red tides: a revisit and newer observations. Estuar Coast Shelf Sci 1989;29:317–326.10.1016/0272-7714(89)90031-0

Catterell WA, Risk M. Toxin T4(6) from *Gymnodinium breve* (formerly *Ptychodiscus brevis*) enhances activation of voltage-sensitive sodium channels by veratridine. Mol Pharmacol 1981;19:345–348. [PubMed: 6262621]

Cheng YS, Zhou Y, Irvin CM, Pierce RH, Naar J, Backer LC, et al. Characterization of marine aerosol for assessment of human exposure to PbTx. Environ Health Perspect 2005;113:638–643. [PubMed: 15866777]

Coleman JR, Ramsdell JS. The type B brevetoxin adversely affects development, cardiovascular function, and survival in medaka embryos-PbTx-3 *Oryzias* research. Environ Health Perspect 2003;111:1920–1925. [PubMed: 14644667]

Douchette, GM.; Kodama, M.; Franca, S.; Gallacher, S. Bacterial interactions with harmful algal bloom species: bloom ecology, toxicology and cytology. In: Anderson, DM.; Cembella, AD.; Hallegraeff,

GM., editors. Psysiological ecology of harmful algal blooms. Springer-Verlag; Heidelberg: 1998. p. 619-647.

Duagbjerg N, Hansen G, Larsen J, Moestrup O. Phylogeny of some of the major genera of dinoflagellates based on ultrastructure and partial LSU r-DNA sequence data: including three new genera of unarmored dinoflagellates. Phycologia 2001;39:302–317.

Epstein, PR. The role of algal blooms in the spread of human cholera. In: Lassus, PG.; Arzul, E.; Gentien, P.; Marcaillou-Le-Baut, C., editors. Harmful marine algal blooms. Lavoisier; Paris: 1995. p. 846

Fleming LE, Kirkpatrick B, Backer LC, Bean JA, Wanner A, Dalpra D, Tamer R, Zaias J, Cheng YS, Pierce R, Naar J, Abraham Clark R, Zhou Y, Henry MS, Johnson D, Van De Bogart G, Bossart GD, Harrington M, Baden DG. Initial evaluation of the effects of aerosolized Florida red tide toxins (PbTx) in persons with asthma. Environ Health Perspect 2005;113:650–657. [PubMed: 15866779]

Flewelling LJ, Naar JP, Abbott JP, Baden DG, Barros NB, Bossart GD, et al. Red tides and marine mammal mortalities. Nature 2005;435:755–756.10.1038/nature435755a [PubMed: 15944690]

Florida Fish and Wildlife Conservation Commission. 2007. web site: www.myfwc.com

Geraci, JR. Final report to the National Marine Fisheries Service. US Navy Office of Naval Research, and Marine Mammal Commission, Ontario Veterinary College, University of Guelph; Guelph, Ontario: 1989. Clinical investigation of the 1987–88 mass mortality of bottlenose dolphins along the US central and south Atlantic coast; p. 1-63.

Guillard, RRL. Division rates. In: Stein, JR., editor. Handbook of phycological Methods. Cambridge University Press; London: 1973. p. 289-311.

Gunter G, Williams RH, Davis CC, Smith FGW. Catastrophic mass mortality of marine animals and coincident phytoplankton bloom on the west coast of Florida, November 1946 to August 1947. Ecol Monogr 1948;18:309–324.10.2307/1948575

Ingersoll E. On the fish mortality in the Gulf of Mexico. US Nat Mus 1882;4:74–80.

Kirkpatrick B, Fleming LE, Backer LC, Bean JA, Tamer R, Kirkpatrick G, et al. Environmental exposures to Florida red tides: effects on emergency room respiratory diagnoses admissions. Harmful Algae 2006;5:526–533.

Landsberg JH. The role of harmful algal blooms in shellfish disease. J Shell Res 1997;16:350.

Landsberg JH. The effects of harmful algal blooms on aquatic organisms. Rev Fisheries Sci 2002;10(2): 113–390.

Landsberg, JH.; Steidinger, KA. A historical review of *Gymnodinium breve* red tides implicated in mass mortalities of the manatee (*Trichechus manatus latirostris*) in Florida, USA. In: Reguera, B.; Blanco, J.; Fernandez, ML.; Wyatt, T., editors. Harmful algae. Xunta de Galicia and Intergovernmental Oceanographic Commission, UNESCO; Paris: 1998. p. 97-100.

Leverone JR, Blake NJ, Pierce RH, Shumway SE. Effects of the dinoflagellate, *Karenia brevis* on larvae; development in three species of bivalve mollusk from Florida. Toxicon 2006;48:75–84. [PubMed: 16814341]

Naar J, Bourdelais A, Tomas C, Kubanek J, Whitney P, Flewelling L, et al. A competitive ELISA to detect PbTx from *Karenia brevis* (formerly *Gymnodinium breve*) in seawater, shellfish and mammalian body fluid. Environ Health Perspect 2002;110:179–185. [PubMed: 11836147]

O'Shea TJ, Rathbun GB, Bonde RK, Buergelt CD, Odell DK. An epizootic of Florida manatees associated with a dinoflagellate bloom. Mar Mamm Sci 1991;7:165–179.10.1111/j.1748-7692.1991.tb00563.x

Pierce, RH.; Henry, MS.; Proffitt, LS.; Hasbrouck, PA. Red tide toxin (brevetoxin) enrichment in marine aerosol. In: Graneli, E.; Sundstrom, B.; Elder, E.; Anderson, DM., editors. Toxic marine phytoplankton. Elsevier; Amsterdam: 1990. p. 397-402.

Pierce, R.; Henry, M.; Blum, P.; Payne, S. *Gymnodinium breve* toxins without cells: intracellular and extracellular toxins. In: Hallegraeff, GM.; Blackburn, SI.; Bolch, CJ.; Lewis, RJ., editors. Harmful algal blooms 2000. IOC of UNESCO; Paris: 2001. p. 421-424.

Pierce RH, Henry MS, Blum PC, Lyons J, Cheng YS, Yazzie D, et al. Brevetoxin concentrations in marine aerosol: human exposure levels during a *Karenia brevis* harmful algal bloom. Bull Environ Contam Toxicol 2003;70:161–165.10.1007/s00128-002-0170-y [PubMed: 12478439]

Pierce, RH.; Henry, MS.; Dickey, R.; Plakas, S. NSP Toxins and metabolites in oysters, clams, and whelks. In: Steidinger, KA.; Landsberg, JH.; Tomas, CR.; Vargo, GA., editors. Harmful algae 2002.

Florida Fish and Wildlife Conservation Commission and International Oceanographic Commission of UNESCO; St. Petersburg, FL: 2004. p. 294-296.

Pierce RH, Henry MS, Blum PC, Hamel SL, Kirkpatrick B, Cheng YS, et al. Brevetoxin composition in water and marine aerosol along a Florida beach: assessing potential human exposure to marine biotoxins. Harmful Algae 2005;4:965–972.10.1016/j.hal.2004.11.004

Plakas SM, El Said KR, Jester ELE, Grande HR, Musser SM, Dickey RW. Confirmation of brevetoxin metabolism in the Eastern oyster (*Crassostrea virginica*) by controlled exposures to pure toxins and *Karenia brevis* cultures. Toxicon 2002;40:721–729.10.1016/S0041-0101(01)00267-7 [PubMed: 12175608]

Plakas SM, Wang Z, El Said K, Jester ELE, Granade HR, Flewelling L, et al. Brevetoxin metabolism and elimination in the Eastern oyster (*Crassostrea virginica*) after controlled exposures to *Karenia brevis*. Toxicon 2004;44:677–685.10.1016/j. toxicon.2004.07.027 [PubMed: 15501294]

Poli MA, Mende TJ, Baden D. PbTx, unique activators of voltage-sensitive sodium channels bind to specific sites in rat synaptosomes. Mol Pharm 1986;1(30):129–135.

Poli MA, Musser SM, Dickey RW, Eilers PP, Hall S. Neurotoxic shellfish poisoning and brevetoxin metabolites: a case study from Florida. Toxicon 2000;38:981–993.10.1016/S0041-0101(99)00191-9 [PubMed: 10728835]

Quick, JA.; Henderson, GE. Effects of *Gynmodinium breve* red tides on fishes and birds: a preliminary report on behavior, anatomy, hematology and histopathology. In: Ambroski, RL.; Hood, MA.; Miller, RR., editors. Proceedings of the Gulf regional symposium on diseases of aquatic animals. Louisiana State University; Louisiana Sea Grant: 1974. p. 85-113.

Rein KS, Baden DG, Gawley RE. Conformational analysis of the sodium channel modulator brevetoxin a, comparison with brevetoxin B conformations, and an hypothesis about the common pharmacophore of the “site 5” toxins. J Org Chem 1994;59:2101–2106.10.1021/jo00087a027

Roberts, BS. Occurrence of *Gymnodinium breve* red tides along the west and east coasts of Florida during 1976 and 1977. In: Taylor, DL.; Seliger, HH., editors. Toxic dinoflagellate blooms. Elsevier North Holland, Inc.; New York: 1979. p. 199-202.

Roberts, BS.; Henderson, GE.; Medlyn, RA. The effects of *Gymnodinium breve* toxin on select mollusks and crustaceans. In: Taylor, DL.; Seliger, HH., editors. Toxic dinoflagellate blooms. Elsevier; New York: 1979. p. 419-424.

Rounsefell, GA.; Nelson, WR. Special Scientific Report No 535. U.S Dept of Interior; 1966. Red tide research summarized to 1964 including an annotated bibliography.

Sayer A, Qing H, Bourdelais AJ, Baden DG, Gibson JE. The effect of brevenal on brevetoxin-induced DNA damage in human lymphocytes. Arch Toxicol 2005;79:683–688.10.1007/s00204-005-0676-2 [PubMed: 15986201]

Shimizu Y, Chou HN, Bando H, VanDuyne G, Clardy JC. Structure of brevetoxin A (gb-1), the most potent toxin in the Florida red tide organism (*Gymnodinium breve*). J Am Chem Soc 1986;108:514–515.10.1021/ja00263a031

Simon JL, Dauer DM. A quantitative evaluation of red tide induced mortalities of benthic invertebrates in Tampa Bay, Florida. Environ Lett 1972;3:229–234. [PubMed: 5079463]

Smayda, TJ. Novel and nuisance phytoplankton blooms in the sea: evidence for a global epidemic. In: Graneli, E.; Sundstrom, B.; Edler, L.; Anderson, DM., editors. Toxic marine phytoplankton. Academic; NewYork: 1990. p. 29-40.

Steidinger, KA.; Baden, DG. Toxic marine dinoflagellates. In: Spector, DL., editor. Dinoflagellates. Academic; New York: 1984. p. 201-299.

Steidinger, KA.; Burklew, M.; Ingle, RM. The effects of *Gymnodinium breve* toxins on estuarine animals. In: Martin, DF.; Padilla, GM., editors. Marine pharmocognosy: action of marine at the cellular level. Academic; New York: 1973. p. 179-202.

Steidinger KA, Roberts BS, Tester PA. Florida red tides. Harmful Algae News 1995;12:1–3.

Taylor HF. Mortality of fishes on the west coast of Florida. Science 1917;45:367–368.10.1126/science.45.1163.367

Tester PA, Steidinger KA. *Gymnodinium breve* red tide blooms: initiation, transport, and consequences of surface circulation. Limnol Oceanogr 1997;42(Part 2):1039–1051.

Tester PA, Turner JT, Shea D. Vectorial transport of toxins from the dinoflagellate *Gymnodinium breve* through copepods to fish. J Plankton Res 2000;22:47–61.10.1093/plankt/22.1.47

Trainer VL, Baden DG. High affinity binding of red tide neurotoxins to marine mammal brain. Aquat Toxicol 1999;46:139–148.10.1016/S0166-445X(98)00125-8

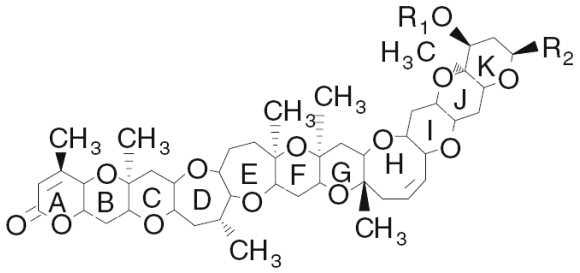
Van Dolah FM. Marine algal toxins: origins, health effects, and their increased occurrence. Environ Health Perspect 2000;108:133–141.10.2307/3454638 [PubMed: 10698729]

Walker ST. Fish mortality in the Gulf of Mexico. Proc US Natl Mus 1884;6:105–109.

Walsh CJ, Luer CA, Noyes DR. Effects of environmental stressors on lymphocyte proliferation in Florida manatees, *Trichechus manatus latirostris*. Vet Immunol Immunopathol 2005;103:247–256.10.1016/j.vetimm.2004.09.026 [PubMed: 15621310]

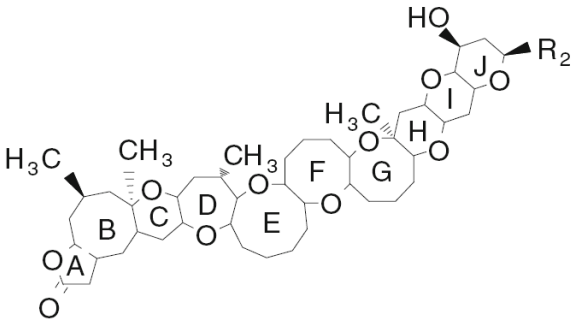
Wang Z, Plakas SM, El Said K, Jester ELE, Granade HR, Dickey RW. LC/MS analysis of brevetoxin metabolites in the Eastern oyster (*Crassostrea virginica*). Toxicon 2004;43:455–465.10.1016/j.toxicon.2004.02.017 [PubMed: 15051410]

Woodcock AH. Note concerning human respiratory irritation associated with high concentrations of plankton and mass mortalities of marine organisms. J Mar Res 1948;7:56–62.



PbTx Type-B

	R1	R2
PbTx-2:	H	$CH_2C(=CH_2)CHO$
PbTx-3:	H	$CH_2C(=CH_2)CH_2OH$
PbTx-5:	CH_3CO	$CH_2C(=CH_2)CHO$
PbTx-6:	H	$CH_2C(=CH_2)CHO$ 27,28 epoxide
PbTx-8:	H	$CH_2C(=CH_2)COCH_2Cl$
PbTx-9:	H	$CH_2CH(CH_3)CH_2OH$



PbTx Type-A

	R1	R2
PbTx-1:	H	$CH_2CH(CH=CH_2)CHO$
PbTx-7:	H	$CH_2CH(CH=CH_2)CH_2OH$
PbTx-10:	H	$CH_2CH(CH_3)CH_2OH$

Fig. 1.
Structures of the two major types of brevetoxin compounds (Poli et al. 1986)

Table 1

Brevetoxin composition (μg/l) and *K. brevis* cell counts (cells/ml) from water samples collected along the south-west Florida coast during the January through February 2005 red tide bloom

Location	PbTx-1	PbTx-2	PbTx-3	Brevenal	Cells/ml
I	1.18 ± 0.89	17.80 ± 10.97	2.24 ± 0.60	0.57 ± 0.45	813 ± 519
II	0.98 ± 1.50	11.31 ± 11.46	0.46 ± 0.70	0.70 ± 0.75	736 ± 682
III	1.09 ± 0.67	15.05 ± 8.31	3.98 ± 3.01	0.91 ± 0.56	1,184 ± 730
IV	0.65 ± 0.93	8.84 ± 10.72	3.96 ± 3.36	0.74 ± 1.10	1,045 ± 1,651
V	1.15 ± 1.41	13.22 ± 8.04	0.49 ± 0.34	1.38 ± 1.50	340 ± 312

I Sarasota Bay; II Tampa to Venice; III Venice to Ft. Myers; IV Ft. Myers to Naples; FL Keys. Mean ± SD

Table 2

Intracellular and extracellular PbTx (μg/l) recovered from *Karenia brevis* blooms in Sarasota Bay, 7/6/05 through 11/3/05

Date	Cells/l	*Karenia brevis*					
		Pbtx-1		Pbtx-2		Pbtx-3	
		Incel	Excel	Incel	Excel	Incel	Excel
7/6/05	357,333	0.12	0.08	1.70	2.49	0.00	1.83
7/11/05	1,404,333	0.24	0.49	3.33	7.76	0.65	0.89
7/18/05	419,333	0.16	0.10	3.46	3.29	0.27	6.23
7/25/05	105,333	0.12	0.00	1.22	0.48	0.00	1.30
8/2/05	8,000	0.00	0.00	0.00	0.31	0.00	1.08
8/18/05	588,000	0.21	0.23	2.98	3.19	0.11	2.26
9/6/05	661,333	0.38	0.10	5.06	1.62	0.12	2.43
9/19/05	545,000	0.24	0.14	4.42	2.42	0.30	3.32
10/4/05	33,333	0.00	0.00	0.08	0.39	0.02	1.97
10/17/05	1,333	0.00	0.00	0.00	0.00	0.00	0.24
11/3/05	0	0.00	0.00	0.00	0.00	0.00	0.23

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Table 3

Brevetoxin composition and *Karenia brevis* cells/l in near shore water (μg/l) and marine aerosol (air)(ng/m^3) samples collected along Siesta Beach, Florida, 3/29 and 3/30/03

Date	Cells/l	*Karenia brevis*					
		Pbtx-1		Pbtx-2		Pbtx-3	
		Water	Air	Water	Air	Water	Air
3/29 am	225,000	0.05	0.15	3.02	9.16	1.18	23.94
3/29 pm	152,000	0.00	0.00	1.69	5.16	1.12	20.21
3/30 am	591,800	0.32	0.00	10.17	1.65	1.40	3.48
3/30 pm	903,170	0.42	0.00	11.04	0.01	2.62	0.54

Table 4

Changes in PbTx-conjugate composition of NSP-contaminated oyster and clam tissue, toxicity by mouse units and *Karenia brevis* cell counts and PbTx-μg/l in ambient water samples, collected before, during and following a red tide bloom in Sarasota Bay, Florida, 1/22/04–4/14/04

Date	Sample	*m/z* 1,018	*m/z* 1,034	*m/z* 990	*m/z* 1,006	MU/100 g	Cells/l	PbTx-μg/l
1/22	Oyst	3.44	0.75	0.54	0.37	<20	5,000	0.20
	Clam	1.87	0.64	0.60	0.40	<20		
2/4	Oyst	12.65	1.31	0.71	nd	32.1	73,000	3.60
	Clam	2.12	0.68	0.64	0.41	<20		
2/18	Oyst	25.78	2.02	1.44	nd	97.7	131,000	3.30
	Clam	4.63	1.48	1.08	0.47	78.2		
3/4	Oyst	12.44	1.36	0.89	nd	35.6	3,000	3.30
	Clam	4.26	1.08	1.01	0.53	66.7		
3/17	Oyst	10.73	1.52	0.97	0.45	22.1	0	0.50
	Clam	3.22	1.51	0.93	0.52	41.7		
3/31	Oyst	4.12	0.90	0.59	nd	<20	0	0.00
	Clam	7.42	1.29	1.15	0.43	<20		
4/14	Oyst	2.75	0.64	0.46	tr	<20	0	0.00
	Clam	2.25	1.21	0.68	0.42	<20		