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Trophic transfer of brevetoxins to the benthic macrofaunal community during a bloom of the harmful dinoflagellate *Karenia brevis* in Sarasota Bay, Florida

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ABSTRACT

Harmful algal blooms can cause mass mortalities of top predators such as fish, marine mammals and seabirds but the food web transfer from toxic phytoplankton to these organisms has not been fully elucidated. Macrobenthic invertebrates in coastal waters, including bivalve suspension- and deposit-feeders, carnivorous gastropods, deposit-feeding amphipods and polychaetes, are a major food source for a wide variety of predators and can thus play a critical role in the trophic transfer of algal toxins to higher trophic levels. The objective of this study was to investigate toxin accumulation in transplanted juvenile hard clams, *Mercenaria mercenaria*, a species naturally occurring in the region, and in various macrobenthic functional groups from Florida coastal waters during a natural bloom of the dinoflagellate, *Karenia brevis*, a producer of brevetoxins. Bloom concentrations in the water column ranged from 100 to 1200 cells ml^{-1} over the course of the experiment. This study revealed that these lipophilic toxins can be rapidly accumulated by both suspension- and deposit-feeding benthos, especially bivalve molluscs [1.9–2.8 μg PbTx-3 eq (g wet weight)$^{-1}$]. Transplanted *M. mercenaria* rapidly accumulated toxins from the water column attaining ~0.5 μg PbTx-3 eq (g wet tissue)$^{-1}$ after only 4 h-exposure to the *K. brevis* bloom and a maximum value of 1.5 ± 0.2 μg PbTx-3 eq (g wet tissue)$^{-1}$ after 72 h. Relatively high brevetoxin concentrations were also measured in co-occurring benthic carnivorous gastropods [1–2.6 μg PbTx-3 eq (g wet weight, WW)$^{-1}$]. Mean toxin concentrations in polychaetes and crustaceans varied in the range ~0.04–0.2 μg PbTx-3 eq (g WW)$^{-1}$ over the study period, and thus were typically lower than in molluscs. This study demonstrated in situ toxin accumulation by benthic primary and secondary consumers during a natural Florida red tide. Accumulation by primary consumers may be highly variable in space and time (as shown in bivalves from the natural benthic community) and among taxonomic groups. Toxin transfer further up the food web will thus depend on the toxin level accumulated in prey, the number of pathways from which the predator may accumulate toxins and on possible biological magnification of lipophilic toxins. Overall, this study revealed qualitatively and quantitatively that benthic consumers of a number of taxa can serve as vectors for transporting brevetoxins within the food web.

1. Introduction

Harmful algal blooms (HABs) caused by proliferation of toxic microalgae are widespread worldwide and involve a variety of causative organisms (cyanobacteria, dinoflagellates, diatoms) and of toxic compounds. Many of these microalgae can cause illness in humans via suspension-feeding shellfish, the primary vectors for poisoning. In the Gulf of Mexico, USA, and most notably along the west coast of Florida, the dinoflagellate *Karenia brevis*, a producer of lipid soluble, cyclic polyether neurotoxins, i.e., brevetoxins (Baden et al., 2005), is responsible for neurotoxic shellfish poisoning (NSP) in humans, primarily via the consumption of contaminated shellfish (e.g., Poli et al., 2000; Watkins et al., 2008). Harmful algae can also be associated with mass mortalities of top predators such as marine mammals (Geraci et al., 1989; Scholin et al., 2000; Flewelling et al., 2005; Fire et al., 2007) and seabirds (Sierra Beltrán et al., 1997; Landsberg, 2002; Shumway et al., 2003), but the links between toxic phytoplankton and these organisms are often poorly understood. Previous studies demonstrated toxin trophic transfer in the pelagic food web from toxigenic phytoplankton to zooplankton (copepods and krill) and to planktivorous fish (e.g., Tester et al., 2000 for brevetoxins; Lefebvre et al., 2002 for domoic acid; Samson et al., 2008 for

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paralytic shellfish toxins, PSTs) suggesting that fish can be a vector for algal toxins to marine mammals and birds. Ingestion of toxic fish was found to be responsible for sea lion mortalities in California (Scholin et al., 2000) and bottlenose dolphin mortalities in Florida (Flewelling et al., 2005); causative toxins were domoic acid produced by the diatom, *Pseudo-nitzschia australis*, and brevetoxins produced by *K. brevis*, respectively. Surprisingly, the dolphin mortality event reported by Flewelling et al. (2005) occurred while only low concentrations of *K. brevis* were detected in the water column. Sampling of live fish in the area 2 wks after the die-off revealed significant levels of brevetoxins in viscera and tissues of planktivorous as well as carnivorous fish.

Although brevetoxins are well known to be icthyotoxic to a number of fish species (e.g., Sievers, 1969; Baden et al., 1979), this does not preclude accumulation of brevetoxins in fish tissues and thus potential vectoring of toxins to higher trophic levels (Tester et al., 2000; Flewelling et al., 2005; Naar et al., 2007). Thus, during a *K. brevis* bloom ≥95% of finfish species collected in Joseph Bay, Florida, including planktivorous, piscivorous species and those feeding on benthic invertebrates, contained detectable brevetoxin levels in muscle and viscera, with consistently higher levels in the latter (Naar et al., 2007). Laboratory experiments found that omnivorous fish accumulating brevetoxins from *Mercenaria mercenaria* prey, showed no adverse effects. Panktivorous fish remained unaffected following consumption of relatively high concentrations of intact *K. brevis* cells, yet experienced high mortalities when exposed to dissolved brevetoxins resulting from cell lysis. Thus, although sensitivity to toxins will vary among and within taxa [e.g., Sievers (1969) for brevetoxins, Bricelj and Shumway (1998) for PSTs], the pathway of exposure will also affect the capacity to accumulate brevetoxins at higher trophic levels. Brevetoxins can also be ingested by herbivorous fish and manatees that feed on benthic seagrasses and their epiphytes coated with toxins released from lysed *K. brevis* cells, which can persist for >10 wks after this species has disappeared from the plankton (Flewelling et al., 2005). These findings suggested post-bloom bioaccumulation processes and imply toxin trophic transfer from both the pelagic and benthic food webs, although much less is known about benthic pathways. Indeed, many carnivorous fish found in Florida waters such as flounders, striped burrfish, longnose killfish, pinfish, spot and bluefish feed on macrobenthic invertebrates (Motta et al., 1995; Luczkovich et al., 2002).

Most macrobenthic invertebrate species are primary consumers and constitute a major food source for a variety of predators. This functional role in the food web may play a critical role in toxin transfer to higher trophic levels. While numerous studies have been conducted on brevetoxin accumulation by commercially exploited suspension-feeding bivalves (e.g., Roberts et al., 1979; Plakas et al., 2008), there is limited information on brevetoxin accumulation in the natural benthic community during a harmful algal bloom (Simon and Dauer, 1972; Summerson and Peterson, 1990; Pierce et al., 2004a). The objective of this study was therefore to investigate toxin trophic transfer from toxic algae to a macrobenthic community from nearshore Florida waters. This was achieved by tracking the brevetoxin body burden of the main taxonomic groups of the natural benthic macrofauna (organisms >1 mm) during a *K. brevis* bloom in February 2005. Brevetoxin accumulation in transplanted juveniles of the suspension-feeding bivalve, *M. mercenaria* (Linnaeus 1758), was followed in parallel for comparison.

2. Materials and methods

2.1. Study site and K. brevis bloom development

The study was conducted in Sarasota Bay (27°N, 82°W), along the southwest coast of Florida, USA. The study area was located on the southeastern section of Longboat Key (Fig. 1). This area consists of a gently sloping bottom of clean, medium to fine quartz sand, with some shell material and no eelgrass or submerged aquatic vegetation (SAV). The experimental study was conducted on duplicate sites located about 300 m apart (salinity = 33). Water column depth in the area was approximately 3.2 m.

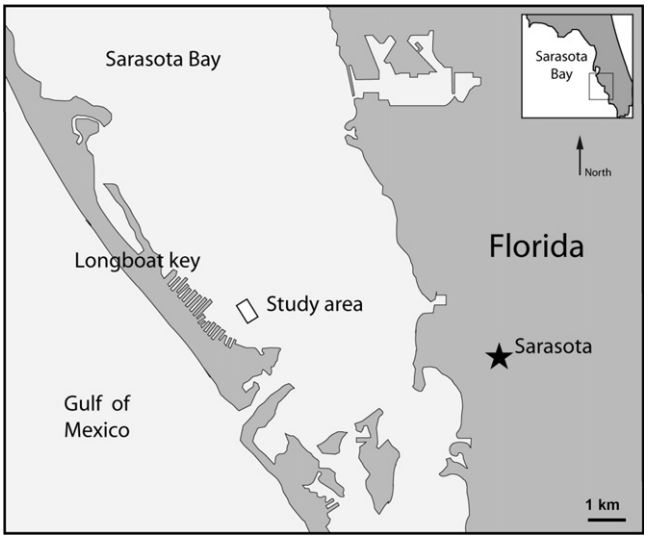

Fig. 1. Map of Sarasota Bay, Florida, USA, and location of study area.

The *K. brevis* bloom started to develop in mid-January 2005. Low cell concentrations were first reported offshore, west of Tampa Bay (NOAA HAB Bulletin, 13 January 2005). The bloom moved south and was located offshore of Sarasota and Venice by January 25. It then extended inshore by February. Respiratory distress in humans and dead fish were reported by the Mote Marine Laboratory at Siesta Key and Longboat Key (NOAA HAB Bulletin, 27 and 31 January 2005). Cell concentrations attained high levels ($>1 \times 10^6$ cells l^{-1}) in Sarasota Bay from February 3 to 10 (Florida Fish and Wildlife Conservation Commission (FWC) HAB Archived Status Maps, 2005). The bloom continued to move south and medium to high concentrations (1×10^5 to $>1 \times 10^6$ cells l^{-1}) were reported in Sarasota counties. The bloom started to weaken by mid-March and very low concentrations ($0–5 \times 10^3$ cells l^{-1}) were reported by late April in the affected area (NOAA HAB Bulletin, 25 April 2005 and Florida FWC). Whole, offshore water samples from the Gulf of Mexico were collected by participants of the Red Tide Offshore Monitoring Program (RTOMP), which includes volunteers, charter boat captains, commercial fishermen, private citizens, divers, and others. The locations of the samples were reported and the samples were delivered to the Florida Fish and Wildlife Research Institute for processing. *K. brevis* cells were identified, counted and reported on the HAB Historical Database and used to generate the Status Maps. More details regarding the history and sampling procedures are described in Heil and Steidinger (2009).

2.2. Brevetoxin accumulation by benthic invertebrates

Juvenile hard clams (=quahogs), *M. mercenaria* (Veneridae), a species naturally found in the study area (16.2 mm shell length ± 1.1 standard deviation, sd) were obtained from a hatchery source (Bay Shellfish Company, Terra Ceia, FL). Clams were maintained in aerated aquaria at 18 °C and supplemented with food (*Isochrysis galbana*) for a week prior to field-deployment in the study area affected by the *K. brevis* bloom. No mortalities were observed among the juveniles prior to the field deployment. Four replicates of 10 individuals were taken to determine initial toxin levels in soft

tissues prior to transfer to the field. For both study sites, 12 trays (15 cm × 15 cm × 10 cm deep) filled with artificial coarse sand and containing 20 clams each were placed on the bottom sediment and covered with a screen (0.5 cm mesh size) to protect clams from predators (crabs and fish). The experiment was conducted from February 8 to February 11. Four trays were collected after 4 h-, 24 h- and 72 h-exposure to the *K. brevis* bloom. Clams were collected, and whole soft tissues were dissected using a scalpel, rinsed with filtered seawater and weighed (wet weight, WW). The 20 individuals from each tray were divided into 2 subsamples of 10 individuals and stored at −80 °C until toxin analysis (each subsample in an individual plastic bag). For data analysis of toxin content, the two subsamples of 10 clams each were averaged and an analysis of variance (ANOVA) was conducted with $n = 4$ samples.

Toxin content of the native benthic community was first determined on February 2 and additional samples were collected on February 8, 9 and 11 (on the same dates as clams). The sediment surface (~5 cm depth) was collected in 20 l buckets by scuba divers, covering about 2 m^2, and sieved on the boat through a 500-μm square mesh to collect benthic macrofauna. In the laboratory, organisms were rinsed with filtered seawater and sorted under a dissecting microscope to separate them into four main taxonomic groups: polychaetes, bivalves, gastropods and crustaceans. As the organisms were relatively small (<1 cm) and none of the species were very abundant (total density of ~21 individuals m^{-2}), all species from each taxonomic group had to be pooled together to obtain sufficient biomass for toxin analysis. Samples obtained from the two study sites were considered as duplicates for data analysis, given that the two sites were very similar in faunal composition (see Section 3). Similar to the processing of the juvenile clams above, the samples were weighed (WW) and stored at −80 °C until toxin analysis. In parallel, the community was sampled at both sites for determination of species composition and community structure. For this, 7 replicate cores (10 cm diameter and 10 cm deep) were taken from each study site. Sediment samples were sieved through a 500-μm square mesh and fixed in 4% formaldehyde.

To monitor and determine *K. brevis* concentration during the study, water samples were collected concurrently from 1 m below the surface using a weighted silicone tube attached to a submersible pump. Samples were preserved using Lugol's iodine solution and counted using a Sedgewick–Rafter chamber visualized on a Zeiss phase-contrast microscope. Water column toxin concentrations (in μg PbTx l^{-1}) at the study site were only measured on February 8 ($n = 5$ consecutive samples over a 1 h-period) by collecting water samples 1 m below the surface and near-bottom at 2.7 m depth, and conducting toxin analysis by liquid chromatography–mass spectrometry (LC–MS) following methods of Pierce et al. (2004b).

2.3. Toxin extraction and analysis

Tissue samples were extracted in 5 ml acetone using a Brinkmann Polytron homogenizer. Slurries were centrifuged (10 min at 3000 rpm) and the supernatant was collected and dried in a rotary-evaporation unit. The dried residues were re-solubilized in acetone at a concentration of 0.1–2 g wet tissue ml^{-1} by sonicating in a bath and vortex mixing.

The tissue toxin content was analysed by competitive Enzyme-Linked Immunosorbent Assay (ELISA) as described in Naar et al. (2002). Due to the small amount of sample, there was not enough to conduct additional confirmatory analysis by chemical methods. Nevertheless, we detemined that the ELISA method would be the most sensitive technique for this study as we anticipated potentially low toxin concentrations in clam tissues. This method allows accurate detection of brevetoxins (PbTxs) in a variety of sample types without matrix effects at a level as low as 4 ng PbTx g^{-1}. The anti-brevetoxin goat polyclonal antibodies used (obtained for this study from J. Naar, University of North Carolina at Wilmington, NC) are specific for the last H-K rings of the PbTx type-2 brevetoxins (PbTx-2, PbTx-3, PbTx-9) and recognize all of these derivatives (Naar et al., 2004; Dickey et al., 2004; Plakas et al., 2004). As ELISA analysis cannot differentiate among the toxin congeners present in the sample tested, results are expressed as PbTx-3 equivalents (eq) and reflect the overall concentration of brevetoxins and brevetoxin-like compounds present in the sample. Statistical analyses were conducted using analysis of the variance (ANOVA).

3. Results

The concentration of *K. brevis* cells in the study area varied between 100 and 1200 cells ml^{-1} near the surface over the course of the bloom (Fig. 2). The highest cell counts were recorded at the beginning of the benthic survey (February 2) and decreased between February 3 and February 7, remaining at a level of ~200 cells ml^{-1} until the end of the study (February 11). On February 8, sampling of water near the bottom (~10 cm off-bottom) showed that cell concentration was much lower at depth with about 75 cells ml^{-1} indicating a low mixing regime of the water column at this time of the study. On this day, when water column toxin concentrations were also measured, these attained a maximum of 30 μg total PbTx l^{-1} at 1 m below the surface, and an order of magnitude lower and more constant levels (~3 μg PbTx l^{-1}) near bottom. Thus *K. brevis* concentrations during the study period consistently remained above 5 cells ml^{-1}, the concentration deemed sufficient to cause suspension-feeding shellfish to become toxic to humans in this region and that leads to closure of shellfish beds in the state of Florida (Pierce and Henry, 2008).

Transplanted *M. mercenaria* (February 8) rapidly accumulated toxins from the water column and experienced no mortalities over the 3-day experiment. The toxin body burden attained ~0.5 μg PbTx-3 eq (g wet tissue)$^{-1}$ ($n = 4$) after only 4 h-exposure to the *K. brevis* bloom (Fig. 3) and increased throughout the experiment to attain a maximum value of 1.5 ± 0.2 μg PbTx-3 eq (g wet tissue)$^{-1}$ after 72 h at site 1. There was a significant effect of both site and time of field exposure on clam toxicity over the entire study period (two-way ANOVA, $p \leq 0.001$), and a non-significant site × time interaction term ($p = 0.14$). Clam toxin accumulation rates were comparable, however, at the two sites during the first day following deployment (1.23 and 1.06 μg g^{-1} PbTx-3 eq day^{-1}).

The natural benthic community was numerically dominated by polychaetes (averaging 89.4 and 87.1% at the two sites sampled). Bivalves represented 5.0–6.1%, crustaceans 3.4–3.0% and gastropods 2.4–2.6% of the total macrofauna. All taxonomic groups except gastropods were dominated by deposit-feeding species.

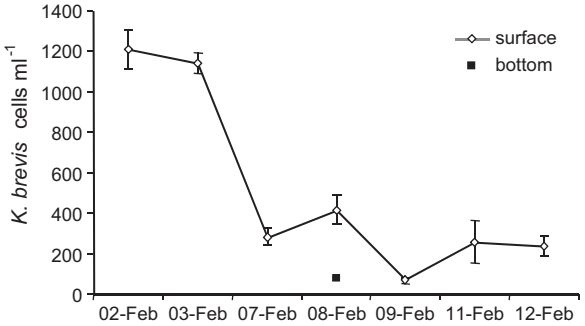

Fig. 2. *Karenia brevis* cell density (mean ± sd, in cells ml^{-1}) in the study area from February 2 to February 12, 2005.

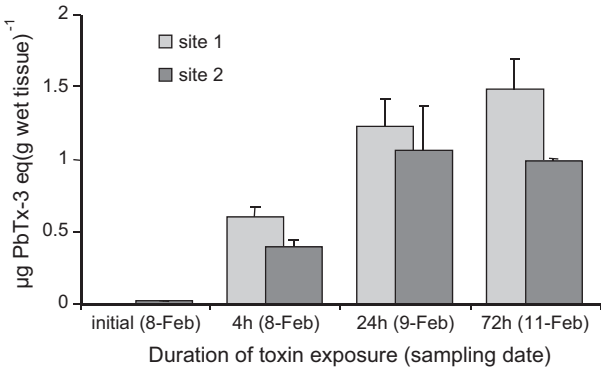

Fig. 3. Brevetoxin concentration in tissues of transplanted juvenile hard clams, *Mercenaria mercenaria* [in µg PbTx-3 eq (g wet tissue)$^{-1}$] after 4 h, 24 h and 72 h exposure to the *Karenia brevis* bloom (n = 4 trays per site and per sampling time).

Polychaetes were largely represented by *Mediomastus ambiseta* (74.4 and 78.2% at each of the two sites), followed by smaller contributions by *Armandia maculata* (6.8–8.6%) and *Prinospio pygmaea* (<5%). The bivalve, *Tellina* spp., represented on average 47.5–52.9% of total bivalve species (n = 7) at the two sites: the two main species present in this geographical area are *T. versicolor* and *T. lineata* (Camp et al., 1998), both deposit-feeders (Arruda et al., 2003). Crustaceans were mainly represented by amphipods and isopods, and the most common species was the amphipod, *Ampelisca abdita* (40–29% of total crustacean species); the isopod, *Edotea montosa* (19.8–13.0%), and two amphipods, a corophid species and *Listriella barnardi* (<17%), made up the next greatest contribution to this taxonomic group. Gastropods were dominated by two carnivore/scavenger species. In particular, *Olivella* sp. represented 81.8 and 75.5% of this taxonomic group at each of the two sites.

Brevetoxins were detected in tissues of all taxonomic groups, but mainly accumulated in bivalves and gastropods, a pattern consistently observed throughout the study period (Fig. 4). Mean toxin concentrations in polychaetes and crustaceans varied in the range ~0.04–0.2 µg PbTx-3 eq (g wet weight)$^{-1}$ over the study period. The mean toxin body burden in gastropods remained around 1 µg PbTx-3 eq (g wet weight)$^{-1}$ from February 2 to February 8 and reached 2.6 ± 1 µg PbTx-3 eq (g wet weight)$^{-1}$ on February 11 (n = 2). Toxin concentrations in bivalves showed high variability between sites, but with no clear tendencies, and with maximal toxin concentrations occurring alternatively at site 1 or site 2. Mean values varied in the range 1.9–2.8 µg PbTx-3 eq (g WW)$^{-1}$,

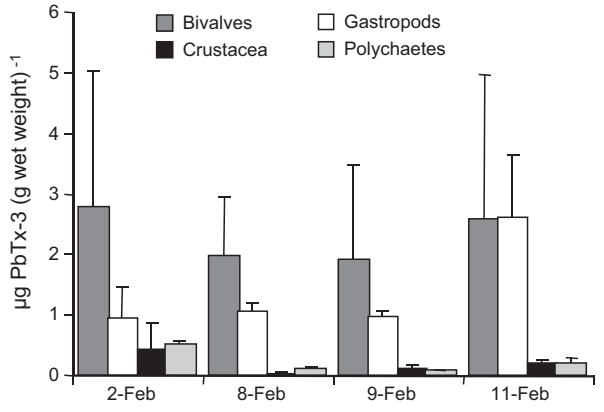

Fig. 4. Brevetoxin concentration [µg PbTx-3 eq (g wet tissue weight)$^{-1}$] in four taxonomic groups of the benthic macrofaunal community in the study area affected by *K. brevis* bloom between February 2 and February 11, 2005. Error bar is standard deviation and n = 2 for all taxonomic groups.

and were thus ca. one or two orders of magnitude higher than those of polychaetes and crustaceans.

4. Discussion

Benthic macrofauna usually constitutes the dominant biomass of primary consumers in marine sediments and plays a major role in food webs. This compartment shows high diversity of feeding modes (suspension- and deposit-feeders, herbivores, omnivores and carnivores) allowing benthic species to feed on a wide variety of organic food sources from both the sediment and the water column. Benthic macrofauna is also characterized by high rates of secondary production and is an important food source for higher trophic levels. When a toxic algal bloom occurs, benthic macrofaunal species can thus play a critical role in the transfer of toxins to higher trophic levels. This will, however, depend on the extent to which species from natural benthic communities are able to accumulate significant toxin levels during a bloom and survive bloom conditions. Toxin uptake in suspension-feeding bivalves has been extensively studied as these organisms are the primary vector of both hydrophilic (Wright et al., 1989; Bricelj and Shumway, 1998) and lipophilic (Watkins et al., 2008) algal toxins to humans via consumption of contaminated shellfish. They can potentially become highly contaminated in a short period of time (hours) due to their ability to concentrate cells by filtering large volumes of water and largely sedentary habit which prevents movement away from affected areas.

Accumulation of brevetoxins by suspension-feeding bivalves has been well established in controlled laboratory studies using *K. brevis* cultures (reviewed by Plakas and Dickey, 2010). These studies include the Eastern oyster, *Crassostrea virginica* (Plakas et al., 2002, 2004; Wang et al., 2004), and Pacific/New Zealand species such as *Crassostrea gigas* (Ishida et al., 2004a), cockles, *Austrovenus stutchburyi* and greenshell mussels, *Perna canaliculus* (Ishida et al., 2004a,b). Few studies have been conducted on the accumulation of PbTxs by bivalves during natural blooms, and these typically rely on collection of specimens at a single time point rather than a time series (e.g., Plakas et al., 2008 for *C. virginica*) and have rarely included *Mercenaria* spp. or reported concurrent *K. brevis* cell densities [although see changes in PbTx-conjugate composition over time in Pierce and Henry (2008)]. Thus, one month following human intoxication via shellfish (whelk) consumption, juvenile *Mercenaria* spp. obtained from Sarasota Bay attained higher toxicities (6.6 µg PbTx-3 g^{-1}) than adult Venus clams, *Chione cancellata* (0.4 µg PbTx-3 g^{-1}) of the same size (~2 mm shell length) and from the same location, using the radioimmunoassay, thus confirming that the former are capable of attaining relatively high PbTx levels (Poli et al., 2000). Both of these bivalve species were deemed likely prey species for whelks in the region. Maximum brevetoxin concentrations of 270 mouse units (MU) 100 g^{-1}, second only to those in "coquinas", *Donax variabilis*, out of seven bivalve species from Florida waters, were reported in *Mercenaria campechiensis* (reviewed by Lansdberg et al., 2009), with a lower maximum of 69 MU 100 g^{-1} for *M. mercenaria* although the latter was measured weeks after the bloom had subsided (Pierce et al., 2004a).

The guidance toxicity level for human consumption (0.8 µg PbTx-3 eq g^{-1}, based on the established mouse toxicity of PbTx-3 = 20 MU 100 g^{-1}) (Naar et al., 2004) was reached after only 24 h-exposure to *K. brevis* bloom at a concentration of 200 cells ml^{-1}, and the toxin body burden was in the range 1–1.5 µg PbTx-3 eq (g wet tissue)$^{-1}$ after 72 h-exposure. Similar brevetoxin concentrations were determined in a tellinid bivalve, *Macoma balthica*, a facultative suspension-feeder, exposed for 24 h to 300–400 *K. brevis* cells ml^{-1} in the laboratory (Haubois et al., 2007). Thus, in contrast to bay scallops, *Argopecten irradians*, that are highly

vulnerable to *K. brevis* blooms (Summerson and Peterson, 1990), results of our study combined with past reports on adult hard clams, confirm that *Mercenaria* sp. can survive bloom conditions and accumulate high brevetoxin concentrations, and can thus potentially vector brevetoxins to higher consumers including crab and fish species. Extensive, species-specific metabolism of brevetoxins to highly persistent cysteine conjugates of variable potency is known to occur in several suspension-feeding bivalves (e.g., Plakas and Dickey, 2010). This can lead to prolonged toxin retention following a *K. brevis* bloom, and a lag time between the end of a bloom and the accumulation of toxins in secondary consumers. Thus, cysteine and cysteine sulfoxide conjugates were present in *M. mercenaria* tissues more than 6 wks after a red tide subsided in Sarasota Bay, in the absence of detectable levels of PbTx-2 (the dominant toxin produced by *K. brevis*) or PbTx-3 (a reduction product in shellfish) (Pierce et al., 2006). It is important to note that the ELISA method used in the present study can detect B-type brevetoxin derivatives, including cysteine conjugates (Plakas and Dickey, 2010).

This study further showed that significant levels of brevetoxins can be accumulated in a natural benthic community dominated by deposit-feeding species. In particular, native bivalves, dominated by deposit-feeding tellinids (*Tellina* spp.), were able to achieve weight-specific brevetoxin body burdens that were comparable with levels attained in the transplanted, suspension-feeding bivalve, *M. mercenaria*, at the same site. Brevetoxin accumulation by a deposit-feeding bivalve, *M. balthica*, was previously demonstrated following laboratory exposure to clay-sedimented *K. brevis* in a study evaluating the impacts of the use of clay flocculation to mitigate HABs (Haubois et al., 2007). In *M. balthica*, feeding on suspended *K. brevis* cells and deposit-feeding on clay-flocculated *K. brevis* yielded comparable toxin levels. The present study demonstrates that brevetoxin transfer to deposit-feeders of various taxonomic groups can also occur under natural bloom conditions. This indicates natural settlement of *K. brevis* cells, coating/adsorption of benthic food sources by brevetoxins released by lysed *K. brevis* cells, and/or possible deposition of zooplankton (e.g., copepod) fecal pellets containing undigested dinoflagellates (Maneiro et al., 2000). The crustacean amphipods, *Ampithoe longimana* and *A. valida*, common herbivores in Florida seagrass beds and an important food source for juvenile fish, readily ingested and accumulated brevetoxins from their own food (freeze-dried seaweed) experimentally coated with *K. brevis* extracts or purified brevetoxins (Sotka et al., 2009). Benthic crustaceans (the barnacle, *Balanus eburneus* and mud crab *Eurypanopeus depressus*) were found to be relatively resistant to the effects of brevetoxins even when exposed to lysed *K. brevis* cells (Sievers, 1969). Additionally, brevetoxins have been detected in surficial natural sediments in nearshore areas affected by *K. brevis* blooms along the southwest Florida coast at levels of up to 3.6 and 9.7 ng g^{-1} dry weight of PbTx-2 and PbTx-3 respectively (Mendoza et al., 2008). Brevetoxins associated with natural coastal sediments as well as with sedimented phosphatic clays used in bloom mitigation (Hitchcock et al., 2012; Pierce et al., 2004b, respectively) can persist for weeks to months following bloom termination and may thus lead to prolonged toxin exposure of deposit-feeding benthos. In our study, detectable brevetoxin accumulation (although at levels much lower than in molluscs), was confirmed in deposit-feeding amphipods and plychaetes (Table 1) in an area with no SAV, pointing to sediments as the primary toxin source. Thus deposit-feeders can constitute a newly described pathway for brevetoxin transfer to higher trophic levels in the natural environment.

In this study, significant toxin concentrations, often exceeding the guidance toxicity level for human consumption, were also measured in carnivorous gastropods that co-occurred and thus potentially preyed on deposit-feeders. Gastropods from the genera *Olivella* and *Acteocina* feed on bivalves, polychaetes and small benthic crustaceans (Chester, 1993; Arruda et al., 2003) and may have accumulated toxins from these sources. Carnivorous gastropods are well known to concentrate PSTs by feeding on bivalves (reviewed by Shumway, 1995). Puffer fish, *Sphaeroides* spp., known to feed on small bivalves and other invertebrates, were also found to concentrate PSTs in Florida waters (Landsberg et al., 2006). Choi et al. (2006), showed experimentally that the gastropod, *Nassarius festivus*, accumulated PSTs when fed with homogenates of contaminated Manila clams (*Ruditapes philippinarum*). Toxin levels in *N. festivus* were 12-fold lower, however, than those measured in its prey. Rapid toxin delivery to the benthos via flocculation of DA-containing *Pseudo-nitzschia* cells in nearshore waters (<15 m) was also shown for four trophic groups in Monterey Bay, CA, including filter-feeders such as sand crabs, predatory flatfish, scavengers such as *Nassarius fossatus*, which attained the highest DA levels, and deposit-feeding ghost shrimp (Goldberg, 2003). In our field study, brevetoxin concentrations recorded in gastropods were about 7 times higher on average than those measured in crustaceans and polychaetes, and reached the level attained in bivalves within this 3-day study with ~2.6 μg PbTx-3 eq g^{-1}. Accordingly, Poli et al. (2000) reported high brevetoxin concentrations in whelks from Sarasota Bay (the predatory gastropod, *Busycon contrarium*) compared to those measured in bivalves, suggesting food web magnification. In contrast to PSTs that are hydrophilic, brevetoxins are lipophilic. Studies on bioaccumulation processes showed that there is a strong correlation between the lipophilicity of organic compounds, degree of bioaccumulation in organisms and biological magnification in food webs (Chessels et al., 1992; Gobas et al., 1993). The tendency of a lipophilic compound to biomagnify in the food web will depend, however, on the balance between its lipophilicity (measured by a partitioning coefficient between water and n-octanol or between lipid and water) and its rate of metabolic transformation and elimination (Gobas et al., 1993). Available data on brevetoxin metabolism and transfer, and on food web transfer of algal toxins in general, are still too scarce to draw definitive conclusions on the potential for biomagnification. Hydrophilic toxins such as domoic acid, have also been shown to accumulate in various food web compartments (copepods, krill, planktivorous fish, crustaceans, cephalopods, seabirds, sea lions, whales) and to cause mass mortalities of top predators (Bargu et al., 2002; Costa et al., 2003; Sierra Beltrán et al., 1997; Scholin et al., 2000). Overall, the ecological impacts of toxin trophic transfer are expected to be strongly related to the potential that a given toxin can be accumulated and magnified at various trophic levels.

Table 1
Relative abundance (mean % of total number of individuals ± standard deviation, sd) and feeding modes of the dominant species comprising the benthic community at the study area. Numerically most abundant species are boldfaced.

Taxonomic group	Species	% ± sd	Feeding mode
Crustaceans	**Ampelisca abdita** (amphipod)	**34** ± 6	Surface deposit-feeder
	Edotea montosa (isopod)	16 ± 5	Omnivore
	Corophiidae (amphipod)	15 ± 12	Surface deposit-feeder
	Listriella barnardi (amphipod)	14 ± 9	(Unknown)
Bivalves	**Tellina spp.**	**50** ± 8	Surface deposit-feeder
	Bivalvia sp. A	21 ± 24	(Unknown)
	Parvilucina multilineata	13 ± 5	Suspension-feeder
Gastropods	**Olivella sp.**	**79** ± 6	Carnivore/scavenger
	Acteocina canaliculata	8 ± 1	Carnivore/scavenger
Polychaetes	**Mediomastus ambiseta**	**76** ± 1	Subsurface deposit-feeder
	Armandia maculata	8 ± 2	(Unknown)

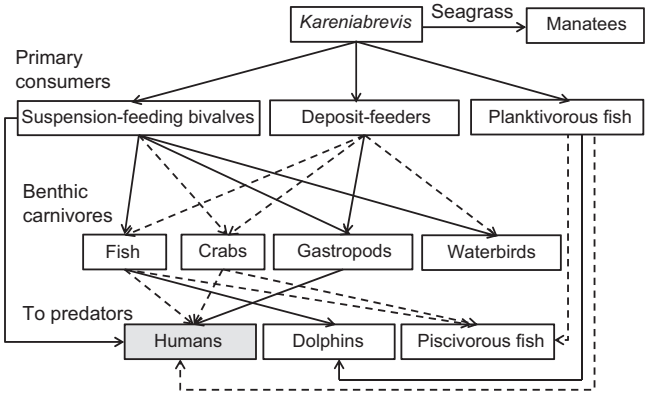

Fig. 5. Trophic pathways for brevetoxins in the Florida marine food web. Black arrows indicate confirmed, quantified links (present study, Tester et al., 2000; Poli et al., 2000; Flewelling et al., 2005; Haubois et al., 2007, and Florida Fish and Wildlife Institute) and interrupted arrows indicate as yet unknown links. Modified from a diagram from Steidinger et al. (2008).

Secondary consumers can thus potentially accumulate brevetoxins from both suspension- and deposit-feeding benthic organisms, which increases the number of pathways for toxins and the risks of toxin accumulation at this trophic level as well as higher up in the food web (Fig. 5). Predators of primary benthic consumers, in addition to gastropods, include aquatic birds, crabs and fish. Diving ducks, oystercatchers, plovers and sandpipers mainly feed on benthic invertebrates. These aquatic birds, as well as crabs, can potentially accumulate toxins through two trophic pathways: directly from benthic primary consumers, and/or via benthic carnivorous gastropods (Forrester et al., 1977; Kreuder et al., 2002). The Fish and Wildlife Research Institute reported mortalities of several thousand diving ducks (the lesser scaup, *Aythya affinis*) during a *K. brevis* bloom in 1974 (see www.floridamarine.org). All digestive tracts of examined birds contained several species of gastropods as well as amethyst gemclams (*Gemma gemma*).

Flewelling et al. (2005) and Naar et al. (2007) showed that omnivorous fish experimentally fed for 2 wks with toxic clams could accumulate brevetoxins in viscera and muscles at levels of 2.6 μg PbTx g^{-1} and 1.5 μg PbTx g^{-1}, respectively. It is now well established that ingestion of fish contaminated with brevetoxins can be responsible for dolphin mortalities (Flewelling et al., 2005), but predatory birds may also be affected via this pathway. Many birds in Florida prey upon fish, including pelicans, herons, egrets, gulls, and cormorants. Certain species of gulls, herons and egrets can also feed on crabs and other benthic invertebrates, increasing the number of possible pathways for brevetoxin accumulation. O'Shea et al. (1991) reported mortalities of doubled-crested cormorants coinciding with manatee mortalities during a *K. brevis* bloom in 1982; however, in general, reports of bird mortalities during HABs are not well documented in the scientific literature (Landsberg et al., 2007).

The finding that brevetoxins can accumulate further up the food chain and thus affect top predators raises concerns about possible alternative pathways toxin transfer to humans other than via commercial bivalves. The only evidence of NSP in humans through consumption of secondary consumers was reported by Poli et al. (2000) for whelks. Fish (flounder, spot, bluefish, pinfish, herrings) and crabs (blue crabs, stone crabs) support important commercial and recreational fisheries in Florida and it would be important to evaluate to what extent they could potentially act as a vector of brevetoxins to humans. So far, there is no evidence that brevetoxin-contaminated fish caused NSP in humans (Naar et al., 2007), Crabs in particular are voracious and very opportunistic predators and can prey upon many benthic invertebrate species as well as on fish carcasses. High concentrations of domoic acid have been detected in the swimming crab, *Polybius henslowii*, along the Portuguese coast (Costa et al., 2003). In Norway, in 2002, several hundred people suffered diarrhetic shellfish poisoning after eating recreationally harvested brown crabs (*Cancer pagurus*) and toxin analysis of crab tissues revealed the presence of okadaic acid and derivatives (Torgersen et al., 2005). There are no reports to date, however, of brevetoxin accumulation in crabs (Roberts et al., 1979).

In conclusion, toxin food web transfer is the result of a complex interaction between physical, biological and chemical processes. Accumulation in primary consumers may be highly variable in space and time (as shown in the present study by the high variability of toxin body burden in bivalves from the benthic natural community) and among taxonomic groups. Toxin transfer further up the food chain will thus depend on the level accumulated in prey, on the number of pathways from which the predator may accumulate toxins and on possible biological magnification. Finally, the fate of toxins in the food web and their potency will depend on their residence time and on their metabolism in both prey and predators.

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References

Arruda, E.P., Domaneschi, O., Amaral, A.C.Z., 2003. Mollusc feeding guilds on sandy beaches in Sao Paulo State, Brazil. Mar. Biol. 143, 691–701.

Baden D.G., Mende T.J., Block, R.E., 1979. Two similar toxins isolated from *Gymnodinium breve*. In: Taylor, D.L., Seliger E.E. (Eds.), Toxic Dinoflagellate Blooms, Proceedings of the Second International Conference on Toxic Dinoflagellate Blooms, Key Biscayne, Florida, Oct. 31–Nov. 5, 1978, Elsevier/North Holland, Inc., pp. 327–334.

Baden, D.G., Bourdelais, A.J., Jacocks, H., Michelliza, S., Naar, J., 2005. Natural and derivative brevetoxins: historical background, multiplicity, and effects. Environ. Health Perspect. 113 (5), 621–625.

Bargu, S., Powell, C.L., Coale, S.L., Busman, M., Doucette, G.J., Silver, M.W., 2002. Krill: a potential vector for domoic acid in marine food webs. Mar. Ecol. Prog. Ser. 237, 209–216.

Bricelj, V.M., Shumway, S.E., 1998. Paralytic shellfish toxins in bivalve molluscs: occurrence, transfer kinetics and biotransformation. Rev. Fish. Sci. 6, 315–383.

Camp, D.K., Lyons, W.G., Perkins, T.H., 1998. Checklist of selected shallow-water marine invertebrates of Florida. Florida Marine Research Institute Technical Report, 239 pp.

Chessels, M., Hawker, D.W., Connel, D.W., 1992. Influence of solubility in lipid on bioconcentration of hydrophobic compounds. Ecotoxicol. Environ. Saf. 23, 260–273.

Chester, C.M., 1993. Comparative feeding biology of *Acteocina canaliculata* (Say, 1826) and *Haminoea solitaria* (Say, 1822) (Opisthobranchia: Cephalaspidea). Am. Malacol. Bull. 10, 93–101.

Choi, M-C., Yu, P.K.N., Hsieh, D.P.H., Lam, P.K.S., 2006. Trophic transfer of paralytic shellfish toxins from clams (*Ruditapes philippinarum*) to gastropods (*Nassarius festivus*). Chemosphere 64, 1642–1649.

Costa, P.R., Rodrigues, S.M., Botelho, M.J., Sampayo, M.A., 2003. A potential vector of domoic acid: the swimming crab *Polybius henslowii* Leach (Decapoda-Brachyura). Toxicon 42, 135–141.

Dickey, R.W., Plakas, S.M., Jester, E.L.E., Elsiad, K.R., Johannessen, J.N., Flewelling, L.J., Scott, P., Hammond, D.G., VanDolah, F.M., Leighfield, T.A., Bottein, Y., Ramsdell, J.S., Busman, M., Moeller, P.D., Pierce, R.H., Henry, M.S., Poli, M.A., Walker, C.S., Kurtz, J., Naar, J.P., Baden, D.G., Musser, S.M., Truman, P., Quilliam, M.A., Stirling, D., Hawryluk, T.P., Wekell, M.M., Hungerford, J.M., Yoshimoto, K., 2004. Multi-laboratory study of five methods for the determination of brevetoxins in shellfish tissue extracts. In: Steidinger, K.A., Landsberg, J.H., Tomas, C.R., Vargo, G.A. (Eds.), Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO, St. Petersburg, FL, pp. 300–303.

Fire, S.P., Fauquier, D., Flewelling, L.J., Henry, M.S., Naar, J.P., Pierce, R.H., Randall, S.W., 2007. Brevetoxin exposure in bottlenose dolphins (*Tursiops truncatus*)

associated with *Karenia brevis* blooms in Sarasota Bay, Florida. Mar. Biol. 152, 827–834.
Flewelling, L.J., Naar, J.P., Abbott, J.P., Baden, D.G., Barros, N.B., Bossart, G.D., Bottein, M.Y.D., Hammond, D.G., Haubold, E.M., Heil, C.A., Henry, M.S., Jacocks, H.M., Leighfield, T.A., Pierce, R.H., Pitchford, T.D., Rommel, S.A., Scott, P.S., Steidinger, K.A., Truby, E.W., Van Dolah, F.M., Landsberg, J.H., 2005. Red tides and marine mammal mortalities. Nature 435, 755–756.
Florida Fish and Wildlife Conservation Commission. Harmful Algal Bloom Archived Status Maps. *Karenia brevis* counts: http://myfwc.com/research/redtide/maps/2005/.
Forrester, D.J., Gaskin, J.M., White, F.H., Thompson, N.P., Quick, J.A., Henderson, G.E., Woodard, J.C., Robertson, W.D., 1977. An epizootic of waterfowl associated with a red tide episode in Florida. J. Wildl. Dis. 13, 160–167.
Geraci, J.R., Anderson, D.M., Temperi, R.J., Aubin, D.J.S., Early, G.A., Prescott, J.H., Mayo, C.A., 1989. Humpack whales (*Megaptera novaeangliae*) fatally poisoned by dinoflagellate toxin. Can. J. Fish. Aquat. Sci. 46, 1895–1898.
Gobas, F.A.P.C., Zhang, X., Wells, R., 1993. Gastrointestinal magnification: the mechanism of biomagnification and food chain accumulation of organic chemicals. Environ. Sci. Technol. 27, 2855–2863.
Goldberg, J.D., 2003. Domoic acid in the benthic fod web of Monterey Bay, California. Master of Science. Moss Landing: California State University Monterey Bay, 41 pp.
Haubois, A.G., Bricelj, V.M., Naar, J.P., 2007. Transfer of brevetoxins to a tellinid bivalve by suspension- and deposit-feeding and its implications for clay mitigation of *Karenia brevis* blooms. Mar. Biol. 151, 2003–2012.
Heil, C.A., Steidinger, K.A., 2009. Monitoring, management and mitigation of *Karenia* blooms in the eastern Gulf of Mexico. Harmful Algae 8, 611–617.
Hitchcock, G.L., Fourquerean, J.W., Drake, J.L., Mead, R.N., Heil, C.A., 2012. Brevetoxin persistence in sediments and seagrass epiphytes of east Florida coatsal waters. Harmful Algae 13, 89–94.
Ishida, H., Nozawa, A., Nukaya, H., Tsuji, K., 2004a. Comparative concentrations of brevetoxins PbTx-2, PbTx-3, BTX-B1 and BTX-B5 in cockle, *Austrovenus stutchburyi*, greenshell mussel, *Perna canaliculus*, and Pacific oyster, *Crassostrea gigas*, involved in neurotoxic shellfish poisoning in New Zealand. Toxicon 43, 779–789.
Ishida, H., Nozawa, A., Nukaya, H., Rhodes, L., McNabb, P., Holland, P.T., Tsuji, K., 2004b. Confirmation of brevetoxin metabolism in cockle, *Austrovenus stutchburyi*, and greenshell mussels, *Perna canaliculus*, associated with New Zealand neurotoxic shellfish poisoning, by controlled exposure to *Karenia brevis*. Toxicon 43, 701–712.
Kreuder, C., Mazet, J.A.K., Bossart, G.D., Carpenter, T.E., Holyoak, M., Elie, M.S., Wright, S.D., 2002. Clinicopathologic features of suspected brevetoxicosis in double-creasted cormorants (*Phalacrocorax auritus*) along the Florida Gulf coast. J. Zoo Wildl. Med. 33 (1), 8–15.
Landsberg, J.H., 2002. The effects of harmful algal blooms on aquatic organisms. Rev. Fish. Sci. 10, 113–390.
Landsberg, J.H., Hall, S., Johannessen, J.N., White, K.D., Conrad, S.M., Abbot, J.P., Flewelling, L.J., Richardson, R.W., Dickey, R.W., Jester, E.L., Etheridge, S.M., Deeds, J.R., Van Dolah, F.M., Leighfield, T.A., Zou, Y., Beaudry, C.g., Benner, R.A., Rogers, P.L., Scott, P.S., Kawabata, K., Wolny, J.L., Steidinger, K.A., 2006. Saxitoxin puffer fish poisoning in the United States, with the first report of *Pyrodinium bahamense* as the putative toxin source. Environ. Health Perspect. 14 (10), 1502–1507.
Landsberg, J.H., Vargo, G.A., Flewelling, L.J., Wiley, F.E., 2007. Algal biotoxins. In: Thomas, N.J., Hunter, D.B., Atkinson, C.T. (Eds.), Infectious and Parasitic Diseases of Wild Birds. Blackwell Press, Oxford, pp. 431–455.
Lansdberg, J.H., Flewelling, L.J., Naar, J.P., 2009. *Karenia brevis* red tides, brevetoxins in the food web, and impacts on natural resources: decadal advancements. Harmful Algae 8, 598–607.
Lefebvre, K.A., Bargu, S., Kieckhefer, T., Silver, M.W., 2002. From sanddabs to blue whales: the pervasiveness of domoic acid. Toxicon 40, 971–977.
Luczkovich, J.J., Ward, G.P., Johnson, J.C., Christian, R.R., Baird, D., Neckles, H., Rizzo, W.M., 2002. Determining the trophic guilds of fishes and macroinvertebrates in a seagrass food web. Estuaries 25, 1143–1163.
Maneiro, I., Frangopulos, M., Guisande, C., Fernández, M., Reguera, B., Riveiro, I., 2000. Zooplankton as a potential vector of diarrhetic shellfish poisoning toxins through the food web. Mar. Ecol. Prog. Ser. 201, 155–163.
Mendoza, W.G., Mead, R.N., Brand, L.E., Shea, D., 2008. Determination of brevetoxin in recent marine sediments. Chemosphere 73, 1373–1377.
Motta, P.J., Clifton, K.B., Hernandez, P., Eggold, B.T., Giordano, S.D., Wilcox, R., 1995. Feeding relationships among nine species of seagrass fishes of Tampa Bay, Florida. Bull. Mar. Sci. 56, 185–200.
Naar, J.P., Bourdelais, A., Tomas, C., Kubanek, J., Whitney, P.L., Flewelling, L.J., Steidinger, K., Lancaster, J., Baden, D.G., 2002. A competitive ELISA to detect brevetoxins from *Karenia brevis* (formely *Gymnodinium breve*) in seawater, shellfish, and mammalian body fluid. Environ. Health Perspect. 110 (2), 179–185.
Naar, J.P., Weidner, A., Kubanek, J., Flewelling, L.J., Bourdelais, A., Jacocks, H., Steidinger, K.A., Pierce, R., Baden, D.G., 2004. Bioaccumulation, biodepuration, and biotransformation of brevetoxins by shellfish: an issue for shellfish monitoring. In: Steidinger, K. (Ed.), Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission and International Oceanographic Commission of UNESCO, pp. 488–490.
Naar, J.P., Flewelling, L.J., Lenzi, A., Abbot, J.P., Granholm, A., Jacocks, H.M., Gannon, D., Henry, M., Pierce, R., Baden, D.G., Wolny, J., Landsberg, J.H., 2007. Brevetoxins, like ciguatoxin, are potent icthyotoxic neurotoxins that accumulate in fish. Toxicon 50, 707–723.
NOAA HAB Bulletin, 13 January 2005. National Oceanic and Atmospheric Administration Harmful Algal Bloom Operational Forecast System Bulletin Archives. http://tidesandcurrents.noaa.gov/hab/bulletins.html.
NOAA HAB Bulletin, 27 January 2005. National Oceanic and Atmospheric Administration Harmful Algal Bloom Operational Forecast System Bulletin Archives. http://tidesandcurrents.noaa.gov/hab/bulletins.html.
NOAA HAB Bulletin, 31 January 2005. National Oceanic and Atmospheric Administration Harmful Algal Bloom Operational Forecast System Bulletin Archives. http://tidesandcurrents.noaa.gov/hab/bulletins.html.
O'Shea, T.J., Rathbun, G.B., Bonde, R.K., Buergelt, C.D., Odell, D.K., 1991. An epizootic of Florida manatees associated with a dinoflagellate bloom. Mar. Mammal Sci. 7, 165–179.
Pierce, R.H., Henry, M.S., Dickey, R., Plakas, S., 2004a. NSP (*Karenia brevis*) toxins and metabolites in oysters, clams and whelks. In: Steidinger, K.A., Landsberg, J.H., Tomas, C.R., Vargo, G.A. (Eds.), Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission and International Oceanographic Commission of UNESCO, pp. 294–296.
Pierce, R.H., Henry, M.S., Higham, C.J., Blum, P., Sengco, M.R., Anderson, D.M., 2004b. Removal of harmful algal cells (*Karenia brevis*) and toxins from seawater culture by clay flocculation. Harmful Algae 3, 141–148.
Pierce, R.H., Henry, M.S., Blum, P.C., Plakas, S.M., Granade, H.R., Jester, E.L.E., El Said, K.R., Dickey, R.E., Steidinger, K.A., Scott, P.S., Flewelling, L.J., Wright, J.L.C., 2006. Comparison of methods for determination of brevetoxins and their metabolites in NSP-toxic bivalved molluscs. In: Henshilwood, K., Deegan, B., McMahon, T., Cusack, C., Keaveney, S., Silke, J., O'Cinneide, M., Lyons, D., Hess, P. (Eds.), Proceedings of the 5th International Conference on Molluscan Shellfish Safety, Galway Ireland, June 14–18, 2004. The Marine Institute, Rinville, Oranmore, Galway, Ireland, pp. 37–42.
Pierce, R.H., Henry, M.S., 2008. Harmful algal toxins of the Florida red tide (*Karenia brevis*): natural chemical stressors in South Florida coastal ecosystems. Ecotoxicology 17, 623–631.
Plakas, S.M., El Said, K.R., Jester, E.L.E., Granade, H.R., Musser, S.M., Dickey, R.W., 2002. Confirmation of brevetoxin metabolism in the eastern oyster (*Crassostrea virginica*) by controlled exposures to pure toxins and to *Karenia brevis* cultures. Toxicon 40, 721–729.
Plakas, S.M., Wang, Z., El Said, K.R., Jester, E.L.E., Granade, H.R., Flewelling, L.J., Scott, P., Dickey, R.W., 2004. Brevetoxin metabolism and elimination in the Eastern oyster (*Crassostrea virginica*) after controlled exposures to *Karenia brevis*. Toxicon 44 (6), 677–685.
Plakas, S.M., Jester, E.L.E., El Said, K.R., Grenade, H.R., Abraham, A., Dickey, R.W., Scott, P.S., Flemwelling, M., Henry, M., Blum, M., Pierce, R., 2008. Monitoring of brevetoxins in the *Karenia brevis* bloom-exposed Eastern oyster (*Crassostrea virginica*). Toxicon 62, 32–38.
Plakas, S.A., Dickey, R.W., 2010. Advances in monitoring and toxicity assessment of brevetoxins in molluscan shellfish. Toxicon 56, 137–149.
Poli, M.A., Musser, S.M., Dickey, R.W., Eilers, P.P., Hall, S., 2000. Neurotoxic shellfish poisoning and brevetoxin metabolites: a case study from Florida. Toxicon 38, 981–993.
Roberts, B.S., Henderson, G.E., Medlyn, R.A., 1979. The effect of *Gymnodinium breve* toxins(s) on selected mollusks and crustaceans. In: Taylor, D.L., Seliger, E.E. (Eds.), Toxic Dinoflagellate Blooms, Proceedings of the Second International Conference on Toxic Dinoflagellate Blooms, Key Biscayne, Florida, Oct. 31–Nov. 5, 1978, Elsevier/North-Holland, Inc., pp. 419–424.
Samson, J.C., Shumway, S.E., Weis, J.S., 2008. Effects of the toxic dinoflagellate, *Alexandrium fundyense* on three species of larval fish: a food-chain approach. J. Fish Biol. 72, 168–188.
Scholin, C.A., Gulland, F., Doucette, G.J., Benson, S., Busman, M., Chavez, F.P., Cordaro, J., DeLong, R., De Vogelaere, A., Harvey, J., Haulena, M., Lefebvre, K., Lipscomb, T., Loscutoff, S., Lowenstine, L.J., Marin III, Miller, P.E., McLellan, W.A., Moeller, P.D.R., Powell, C.L., Rowles, T., Silvagni, P., Silver, M., Spraker, T., Trainer, V., Van Dolah, F.M., 2000. Mortality of sea lions along the central California coast linked to a toxic diatom bloom. Nature 403, 80–84.
Shumway, S.E., 1995. Phycotoxin-related shellfish poisoning: bivalve molluscs are not the only vectors. Rev. Fish. Sci. 3, 1–31.
Shumway, S.E., Allen, S.M., Boersma, P.D., 2003. Marine birds and harmful algal blooms: sporadic victims and under-reported events? Harmful Algae 2, 1–17.
Sierra Beltrán, A., Palafox-Uribe, M., Grajales-Montiel, J., Cruz-Villacorta, A., Ochoa, J.L., 1997. Sea bird mortality at Cabo San Lucas, Mexico: evidence that toxic diatom blooms are spreading. Toxicon 35, 447–453.
Simon, J.L., Dauer, D.M., 1972. A quantitative evaluation of red-tide induced mass mortalities of benthic invertebrates in Tampa Bay, Florida. Environ. Lett. 3 (4), 229–234.
Sievers, A.M., 1969. Comparative toxicity of *Gonyaulax monilata* and *Gymnodinium breve* to annelids, crustaceans, molluscs and a fish. J. Protozool. 16 (3), 401–404.
Steidinger, K.A., Landsberg, J.H., Flewelling, L.J., Kirkpatrick, B.A., 2008. Toxic dinoflagellates. In: Walsh, J., Smith, S.L., Fleming, L.E., Solo-Gabriele, H., W.H. Gerwick, W.H. (Eds.), Oceans and Human Health: Risks and Remedies from the Sea. Elsevier Science Publishers, New York, pp. 239–256.
Sotka, E.E., McCarty, A., Monroe, E.A., Oakman, N., Van Dolah, F.M., 2009. Benthic herbivores are not deterred by brevetoxins produced by the red tide dinoflagellate *Karenia brevis*. J. Chem. Ecol. 35, 851–859.
Summerson, H.C., Peterson, C.H., 1990. Recruitment failure of the bay scallop, *Argopecten irradians concentricus*, during the first red tide, *Ptychodiscus brevis*, outbreak recorded in North Carolina. Estuaries 13 (3), 322–331.
Tester, P.A., Turner, J.T., Shea, D., 2000. Vertical transport of toxins from dinoflagellate *Gymnodinium breve* through copepods to fish. J. Plankton Res. 22 (1), 47–61.

Torgersen, T., Aasen, J., Aune, T., 2005. Diarrhetic shellfish poisoning by okadaic acid esters from brown crabs (*Cancer pagurus*) in Norway. Toxicon 46, 572–578.

Wang, Z., Plakas, S.M., El Said, K.R., Jester, E.L.E., Granade, H.R., Dickey, R.W., 2004. LC/MS analysis of brevetoxin metabolites in the Eastern oyster (*Crassostrea virginica*). Toxicon 43, 455–465.

Watkins, S.M., Reich, A., Flemling, L.E., Hammond, R., 2008. Neurotoxic shellfish poisoning. Mar. Drugs 6, 431–455.

Wright, J.L.C., Boyd, R.K., de Freitas, A.S.W., Falk, M., Foxall, R.A., Jamieson, W.D., Laycock, M.V., McCulloch, A.W., McInnes, A.G., 1989. Identification of domoic acid, a neuroexcitatory amino acid, in toxic mussels from eastern Prince Edward Island. Can. J. Chem. 67, 481–490.