J Chem Ecol (2009) 35:851–859
DOI 10.1007/s10886-009-9658-9

Benthic Herbivores are not Deterred by Brevetoxins Produced by the Red Tide Dinoflagellate *Karenia Brevis*

Erik E. Sotka · Amanda McCarty · Emily A. Monroe · Nicole Oakman · Frances M. Van Dolah

Received: 20 April 2009 / Revised: 12 June 2009 / Accepted: 18 June 2009 / Published online: 11 July 2009

Abstract Gulf of Mexico blooms of the dinoflagellate *Karenia brevis* produce neurotoxic cyclic polyethers called brevetoxins. During and after a red tide bloom in southwestern Florida, *K. brevis* cells lyse and release brevetoxins, which then sink to the benthos and coat the surfaces of seagrasses and their epiphytes. We tested the possibility that these brevetoxin-laden foods alter the feeding behavior and fitness of a common benthic herbivore within Floridean seagrass beds, the amphipod *Ampithoe longimana*. We demonstrated that coating foods with *K. brevis* extracts that contain brevetoxins at post-bloom concentrations (1 μg g^{-1} drymass) does not alter the feeding rates of Florida nor North Carolina populations of *A. longimana,* although a slight deterrent effect was found at eight and ten-fold greater concentrations. During a series of feeding choice assays, *A. longimana* tended not to be deterred by foods coated with *K. brevis* extracts nor with the purified brevetoxins PbTx-2 and PbTx-3. Florida juveniles isolated with either extract-coated or control foods for 10 days did not differ in survivorship nor growth. A similar lack of feeding response to brevetoxin-laden foods also was exhibited by two other generalist herbivores of the southeastern United States, the amphipod *A. valida* and the urchin *Arbacia punctulata*. Given that benthic mesograzers constitute a significant portion of the diet for the juvenile stage of many nearshore fishes, we hypothesize that the ability of some mesograzers to feed on and retain brevetoxins in their bodies indicates that mesograzers may represent an important route of vertical transmission of brevetoxins through higher trophic levels within Gulf of Mexico estuaries.

Keywords Herbivory · Harmful algal bloom · Seagrass · Epiphytic algae · Benthic food web

E. E. Sotka (✉) · A. McCarty · N. Oakman
Grice Marine Laboratory and Department of Biology,
College of Charleston,
205 Fort Johnson Road,
Charleston, SC 29412, USA
e-mail: SotkaE@cofc.edu

E. A. Monroe · F. M. Van Dolah
Marine Biotoxins Program,
National Oceanographic and Atmospheric Administration,
Center for Coastal Environmental Health and Biomolecular Research,
Charleston, SC, USA

E. A. Monroe · F. M. Van Dolah
Marine Biomedicine and Environmental Sciences,
Medical University of South Carolina,
Charleston, SC, USA

Introduction

Gulf of Mexico blooms of the planktonic dinoflagellate *Karenia brevis* are among the oldest reported harmful algal blooms known (Landsberg et al. 2005), and some evidence indicates that their blooms have increased in both frequency and severity over the past 50 years (Alcock 2007). In the eastern Gulf of Mexico, *K. brevis* blooms typically originate on the west Florida shelf and are carried into coastal waters by prevailing wind and currents (Steidinger et al. 1998). Coastal blooms often are maintained in nearshore waters and shallow-water estuaries for months before subsiding, and thus, can have extensive impact on pelagic and benthic communities across large spatial scales (Tester and Steidinger 1997). The primary ecological impacts associated with *K. brevis* blooms are profound mortality events involving finfish, birds, and mammals (Van Dolah et al. 2001; Flewelling et al. 2005). The toxic

effects of *K. brevis* are due largely to brevetoxins, lipophilic cyclic polyethers that bind to and activate voltage-dependent sodium channels (VDSC), and thereby disrupt regulation of respiration and circulation (Baden 1989; Baden et al. 2005).

Adverse effects of brevetoxins can occur after either direct inhalation or consumption of brevetoxin-laden prey. Although distinguishing the relative importance of inhalation vs. consumption can be difficult, there is evidence that vertical transmission via consumption plays a primary role (Poli et al. 2000; Tester et al. 2000; Van Dolah et al. 2001; Doucette et al. 2005; Fire et al. 2008). In the pelagos, *K. brevis* cells commonly are consumed by zooplanktivorous herbivores (e.g., Naar et al. 2007), even though *K. brevis* is a relatively poor-quality food (Turner and Tester 1997; Tester et al. 2000; Prince et al. 2006) and alters copepod fitness (Breier and Buskey 2007) and swimming behaviors (Cohen et al. 2007). These zooplankton then are consumed by fishes that can accumulate brevetoxins within their own tissues.

On the benthos, brevetoxins are known to pass through benthic food webs via two trophic pathways (see reviews by Van Dolah et al. 2001; Doucette et al. 2005). Filter-feeding bivalves readily consume *K. brevis* cells and accumulate brevetoxins without increasing their mortality rates, thereby facilitating transmission to bivalve consumers such as omnivorous gastropods, fishes, and crabs. In addition, herbivorous fishes and manatees directly consume seagrasses and epiphytes that are coated with brevetoxins as dying cells sink to the benthos, break open, and release brevetoxins. Brevetoxin loads on these benthic plants can be substantial. For example, after a 2002 bloom in southwestern Florida, brevetoxin loads on seagrasses averaged approximately 1 μg g^{-1} drymass, and reached 3.1 μg g^{-1} drymass (Flewelling et al. 2005). The majority of this brevetoxin (~85%) coated epiphytic algae and persisted for more than ten weeks after *K. brevis* cells disappeared. Brevetoxin accumulation on seagrasses and their epiphytes subsequently has been found to be a widespread and common occurrence in southwest Florida during and after *K. brevis* blooms (Flewelling 2008; L. Flewelling, personal communication).

We propose a third pathway by which brevetoxins may be transmitted through benthic food webs, but that has received relatively little attention. Small benthic herbivores known collectively as mesograzers (e.g., amphipods, isopods, and polychaetes; cf. Brawley 1992) live and feed on brevetoxin-laden seagrass and epiphyte tissue, accumulate the toxins, and subsequently transfer brevetoxins to their own predators. Mesograzers are the primary consumers of seagrass epiphytes in many estuarine systems (Jernakoff et al. 1996; Hughes et al. 2004), tend to live several months during which they could potentially accumulate brevetoxin after a bloom relaxes, and are among the predominant prey items of omnivorous and carnivorous fishes, crabs, and shrimps in seagrass beds proximal to the Gulf of Mexico (e.g., Darnell 1961; Virnstein 1987; Motta et al. 1993; Luczkovich et al. 2002). Importantly, juvenile stages of several fish species depend almost entirely on a mesograzer diet (Stoner 1979), thus increasing the likelihood that mesograzers may serve as an important brevetoxin vector in estuarine food webs.

An untested assumption underlying this hypothesis is that benthic herbivores are willing to consume brevetoxin-laden foods. To address this, we pursued a series of feeding and performance assays with the herbivorous amphipod *Ampithoe longimana*, one of the most common herbivores in seagrass beds of the southeastern United States (Nelson 1980; Brooks and Bell 2001; A. McCarty and E. Sotka, personal observation), and we focused on the following questions: First, do amphipods alter their feeding behavior when offered foods coated with *K. brevis* chemical extracts or purified brevetoxin? Second, is amphipod survivorship or growth altered when isolated with foods coated with brevetoxin-laden extracts or with seawater containing solubilized brevetoxins? Third, do amphipods that are continuously fed brevetoxin-laden tissues for days contain measurable levels of toxin? Finally, do other benthic herbivores (the amphipod *Ampithoe valida* and the purple sea urchin *Arbacia punctulata*) show similar feeding responses toward *K. brevis* extracts similar to *A. longimana* ? Our results generally support the notion that benthic herbivores like *A. longimana* readily consume brevetoxin-laden foods, and indicate that benthic mesograzers could serve as important vectors for brevetoxin transmission through estuarine food webs.

Methods and Materials

Collection and Preparation We collected the amphipod *A. longimana* on the west coast of Florida (Tampa Bay 27° 45′ N, 82° 36′ W), the east coast of Florida (Fort Pierce 27° 28′ N, 80° 17′ W), and Bogue Sound, North Carolina (34° 41′ N, 76° 41′ W). In Tampa Bay, amphipods were collected at Lassing Park within seagrass beds of *Thalassia testudinum* and *Halodule wrightii*. Fort Pierce amphipods were collected on the seaweeds *Caulerpa sertulariodes*, *Padina gymnospora*, and *Hincksia feldmania* or in seagrass beds of *Thalassia testudinum* and *Halodule wrightii* at Stan Blum Boat Launch or Jaycee Park. In Bogue Sound, *A. longimana* and *A. valida* were collected at Radio Island Jetty largely from the seaweed *Sargassum filipendula*. The urchin *Arbacia punctulata* was collected from floating docks within Bogue Sound.

All animals were brought to the Grice Marine Laboratory in Charleston, SC, and kept in plastic tubs that

contained unfiltered seawater from Charleston Harbor at ~20°C with a salinity of 26–30 ppt. The water was aerated constantly, and changed approximately once a week. All amphipods were raised on a varied diet of the seaweeds *Sargassum filipendula*, *Hypnea musciformis, Gracilaria tikvahiae,* and *Ulva* (syn. *Enteromorpha*) *intestinalis* collected from Bogue Sound, NC. All seaweeds were washed in freshwater for at least two 30 s intervals in order to eliminate any *Ampithoe longimana* existing on the plants. Cultures were allowed to breed for one to several generations before the experiments began.

The *Ulva intestinalis* tissues used in feeding assays were collected at Radio Island Jetty. The seaweed was rinsed, cleaned of all other biota, and frozen. The frozen alga then was lyophilized at Hollings Marine Laboratory in Charleston, SC, ground into a fine powder with a Wiley mini-mill, and stored at −20°C until use.

A crude brevetoxin extract was prepared from a *K. brevis* Wilson isolate by using a standard solid-phase SPEC extraction (Twiner et al. 2007). In brief, 200 ml of *K. brevis* culture were applied to a pretreated SPEC C18SAR disc (Varian Chromatography, Lake Forest, CA, USA). After a wash with 60 ml of Milli-Q water and 5 min of drying, brevetoxins were eluted from the discs with 20 ml methanol. A total of four extracts were produced from the same culture. The solution concentrations were quantified by using mass spectrometry (Twiner et al. 2007) and an ELISA antibody protocol (Maucher et al. 2007), which has an estimated detection limit of 100 pg / ml. Approximately 97% of the brevetoxins within these extracts was PbTx-1 and PbTx-2, as identified by liquid chromatography / mass spectrometry (following Wang et al. 2004; Zhihong Wang, unpublished data). Purified PbTx-2 and PbTx-3 were obtained from World Ocean Solutions, Wilmington, NC, USA; PbTx-1 was unavailable.

Feeding Assays Assays were performed with *K. brevis* lipophilic extracts that contained brevetoxins or purified brevetoxins. We never offered live *K. brevis* cells because to our knowledge, the cells rarely, if ever, accumulate on seagrass and its epiphytes. We assessed whether *K. brevis* extracts altered herbivore feeding rates by isolating *Ampithoe longimana* individuals on a single food (i.e., a no-choice assay) that was coated with extracts at known concentrations of crude brevetoxin (0.1, 1, and either 8 or 10 μg g^{-1} drymass, depending on the assay) or with ether only. To prepare the brevetoxin-laden food, the correct volume of brevetoxin in methanol was dried by using nitrogen gas, then dissolved in 20 ml of anhydrous ethyl ether and mixed with 1 g of freeze-dried and powdered *Ulva intestinalis*. Ether was removed under rotary evaporation. A solution of 0.18 g agar in 5 ml distilled water was heated to boiling in a microwave oven, cooled, and mixed with 1 g of brevetoxin-coated *Ulva* powder added to 4 ml water. The 10 ml mixture then was poured over window screen and flattened between two pieces of wax paper. After cooling for 30–60 min, the mesh was cut into grids of 6X10 cells (approximately 2 cm^2) and offered to individual amphipods in plastic cups that contained 50 ml of seawater. Ether-only control foods were prepared by rotary-evaporating 20 ml of anhydrous ethyl ether from 1 g of *Ulva* tissue as above. Twenty amphipods from each population were isolated individually with each treatment or control, and placed in the dark at 25°C for approximately 48 h. Previous work had indicated that feeding rates in the dark were higher relative to daytime feeding rates (Amanda McCarty, unpublished data). The number of squares consumed was recorded for all replicates, and a series of nonparametric Kruskal-Wallis tests were performed by using *R* (http://www.r-project.org/) to assess overall differences in feeding rate between control and brevetoxin-coated foods. *Post-hoc* differences were assessed with a series of nonparametric pairwise Wilcoxsan tests.

We assessed whether feeding preference was altered by the presence of crude brevetoxin extracts by offering amphipods a choice of *Ulva* tissue coated with extracts at known concentrations of brevetoxin or with ether only. Foods were created as described for no-choice assays. One 6×5 grid of the brevetoxin treatment (at either 1 or 8 μg g^{-1} drymass) and one control grid were offered to amphipods placed individually in small plastic cups (N=40). For each replicate, the number of cells cleared of food was recorded twice a day, and a replicate was removed from the assay when either nine cells had been removed from either the treatment or the control, or when the sum total of cells removed was ten or more. For each assay, a paired *t*-test was performed by using *R* to determine whether control and treatment foods were differentially consumed.

The application of heated agar did not appear to change the structure of brevetoxins used to produce the test foods, which is consistent with their description as heat-stable toxins (Baden et al. 2005). When heated agar was applied to several samples of purified PbTx-2 and PbTx-3, LC-mass spectrometry detected only these two compounds and a methanol adduct of PbTx-2 that arises from reaction with methanol. There was no detectable open A ring PbTx- 2, 3, oxidized PbTx -2, or open A ring oxidized PbTx-2 (Zhihong Wang, personal communication).

Performance Assay We also assessed the effects of brevetoxin consumption on the survivorship and growth of Florida *A. longimana*. Twenty females carrying eggs were isolated in Petri dishes and allowed time to release their offspring. The mother was removed after the first recorded appearance of juvenile amphipods. The juvenile amphipods were allowed approximately two weeks to grow on a mixed diet of *Sargassum* and *Ulva* before the performance assay began. A dash of cetyl alcohol powder was placed within

each dish to minimize the risk of juvenile amphipods being trapped in surface tension. After two weeks, the juveniles were isolated into separate Petri dishes and allowed one week to feed on fresh *Ulva* collected in Charleston, SC. The amphipods were then placed separately in plastic cups and raised on freeze-dried *Ulva* coated with or without extracts containing 8 μg/g brevetoxin. Because cetyl alcohol powder may preferentially bind to this lipid (T. Leighfield, personal communication), the powder was not added to these experimental cups. The assay was performed on eight juveniles from the Ft. Pierce population and 50 juveniles from the Tampa Bay population, divided evenly between the control and the treatment (n=29). Low numbers of the Ft. Pierce population is attributable to availability in our cultures. The food was prepared as described above in *Feeding Assays* and food and water were changed on day four and eight. The assay persisted for 10 days and was monitored daily for mortality.

To assess growth, pictures of each living amphipod were taken with a Nikon camera at the start of the assay and again at the end of the assay. In each picture, head height was measured at the end of the assay by using ImageJ (http://rsbweb.nih.gov/ij/) and corrected for degree of image magnification. Treatment effects on head height were assessed via an unpaired t-test. Because no females dropped their eggs in either control or treatment groups, we could not assess female fecundity. At the completion of the assay, 13 amphipods from the brevetoxin and control groups were flash-frozen and preserved at −80°C. To confirm that our feeding assays succeeded in exposing these amphipods to brevetoxins, the concentration of brevetoxin in these amphipods then was determined via ELISA. Because the small size of amphipods precluded ELISA analysis of individuals, we grouped one to five amphipods per replicate vials (N=4 and N=3 for brevetoxin and control groups, respectively).

Because brevetoxins can persist in the water column for weeks after a *K. brevis* bloom terminates, we tested the possibility that *A. longimana* amphipod survivorship would be affected by solubilized purified brevetoxins at one and 10 ng / ml. These concentrations were taken from levels recorded during and after a 2002 *K. brevis* bloom in Tampa Bay (Flewelling et al. 2005). However, brevetoxin concentrations in the water column are notoriously variable. For example, during the middle of a 2006 *K. brevis* bloom in northwestern Florida, Naar et al. (2007) measured brevetoxin concentrations in the water column up to ~80 ng / ml. Tester et al. (2008) similarly recorded vertically and horizontally variable brevetoxin concentrations from non-detect to 72 ng/ml in transects through blooms in southwest Florida. Thus, our survivorship assay mimics bloom conditions, but does not assess amphipod response to maximal concentrations that are sometimes found during exceptional blooms. Individual amphipods were isolated within 50 ml seawater inoculated with methanol (at 0.05% concentration), for a final concentration of 0, 1, and 10 ng/ml solubilized purified brevetoxin. We used 2:1 ratio of purified PbTx-2 to PbTx-3, which approximates ratios measured in senescing blooms (Tester et al. 2008). The sample sizes at the inception of the experiment were 32, 26, and 22 for the 0, 1, and 10 ng / ml treatments, respectively. Mortality was assessed every one to four days for 10 days, and analyzed via a χ^2 test.

Results

In a series of no-choice feeding assays, *Ampithoe longimana* individuals from three locations (St. Petersburg and Fort Pierce, Florida and Morehead City, North Carolina) consumed statistically equivalent amounts of control foods or foods coated with *K. brevis* extracts at brevetoxin concentrations of 0.1 and 1 μg g^{-1} (Fig. 1). Given that the brevetoxins persisted on epiphytes scraped from seagrass blades at concentrations up to 3 μg g^{-1} (Flewelling et al. 2005), these results indicate that observed post-bloom concentrations of brevetoxin do not tend to alter feeding rates of this benthic herbivore. At concentrations several-fold greater than observed post-bloom concentrations (i.e., 8 and 10 μg g^{-1}), there was a tendency for Florida and North Carolina individuals to feed at lower rates on foods coated with *K. brevis* extracts relative to control foods. However, the inhibitory effect of the *K. brevis* extract on Florida populations was not consistent across populations. For example, the St. Petersburg population fed significantly less on foods coated with 10 μg g^{-1} during one assay (Fig. 1a), but showed no tendency for lower feeding rates when foods were coated at 8 μg g^{-1} during an assay performed several months later (Fig. 1b).

When offered a choice between control foods or extract-coated foods with brevetoxin concentrations of 1 μg g^{-1}, all three populations of *A. longimana* amphipods consumed significantly more control foods (Fig. 2). Surprisingly, however, when the assay was repeated two days later at a higher concentration (8 μg g^{-1}), Florida populations fed on control and extract-coated foods at statistically equivalent rates. A likely explanation for inconsistent feeding deterrency in no-choice (Fig. 1) and choice (Fig. 2) assays using *K. brevis* extracts is the presence of unstable deterrent compounds that elute with 100% methanol and that are unrelated to the brevetoxins. We, therefore, tested the effects of purified brevetoxins on *A. longimana* feeding behavior by offering two Florida populations feeding choices between foods coated with PbTx2 or PbTx3 at 1 μg g^{-1} and control foods. Amphipods did not discriminate between control and PbTx2-coated foods, and significantly preferred the PbTx3-coated to control foods (Fig. 3).

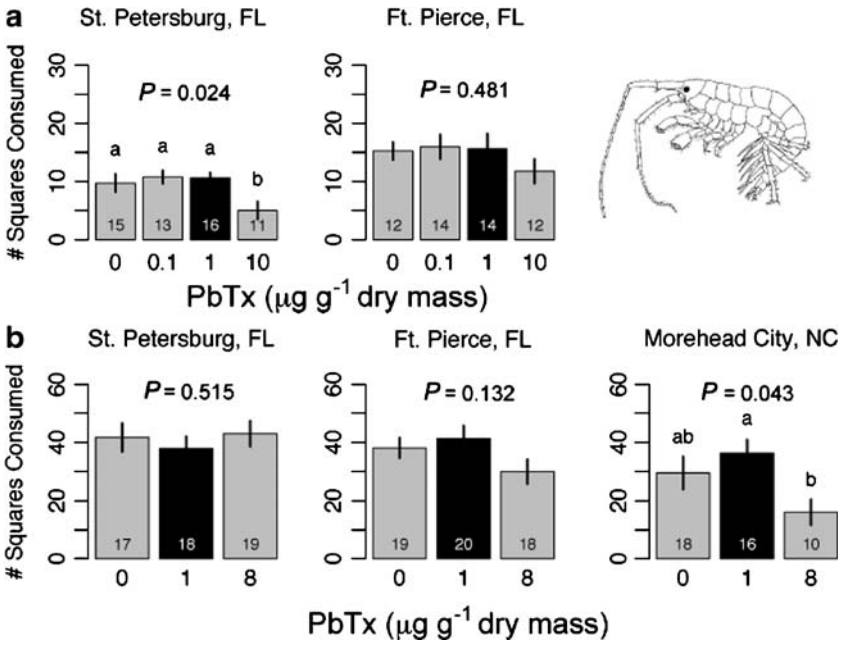

Fig. 1 No-choice feeding assays in which the lipophilic extract of *Karenia brevis* was offered to populations of the herbivorous amphipod *Ampithoe longimana*. Individual amphipods were isolated on freeze-dried *Ulva* (syn. *Enteromorpha*) *intestinalis* tissue coated with ether only ("0"), or with *K. brevis* extracts at a concentration of 1, 8, or 10 μg brevetoxin (g^{-1} dry mass). The *P*-values for Kruskal-Wallis non-parametric tests are indicated, and letters indicate treatments that are statistically indistinguishable by a pairwise Wilcoxan tests. **a**) and **b**) represent assays on independent *K. brevis* extracts and amphipod cultures. The dark bars (1 μg g^{-1}) represent observed concentrations of brevetoxins after bloom events in western Florida

In order to assess whether consuming *K. brevis* extracts has consequences for the survivorship and growth of *A. longimana*, we isolated individual juveniles with foods coated with extracts at 8 μg g^{-1} brevetoxin concentration or control foods. After 10 days, survivorship of Florida *A. longimana* did not differ between treatments (Fig. 4a: χ^2 test: $P>0.100$), nor did size [Mean growth in head height ± S.E. on control foods (0.113±0.021 mm) and extract-coated foods (0.135±0.023 mm); unpaired t-test $P=0.488$)].

To confirm that our feeding assays succeeded in exposing these amphipods to brevetoxins, we froze the juveniles at the end of the performance assay and measured brevetoxin loads within tissues. Juveniles fed brevetoxin-coated foods contained between 2–6 ng g^{-1} wetmass of brevetoxin ($N=4$; data not shown), while juveniles without foods had no detectable brevetoxins ($N=3$). Because individual amphipods averaged 2.8 mg wet mass, each amphipod contained approximately 0.01 ng brevetoxin. Our methods did not distinguish whether these brevetoxins were held in the gut, amphipod tissue, or both.

We also tested whether there was significant mortality when adult amphipods were exposed to seawater with observed concentrations of solubilized brevetoxin (1 or 10 ng ml^{-1}). After 10 days of exposure, control and brevetoxin-laden waters did not alter the survivorship of adult amphipods (Fig. 4b; χ^2 test: $P>0.100$).

To assess whether the lack of a feeding response to brevetoxin-coated foods is common to other generalist benthic

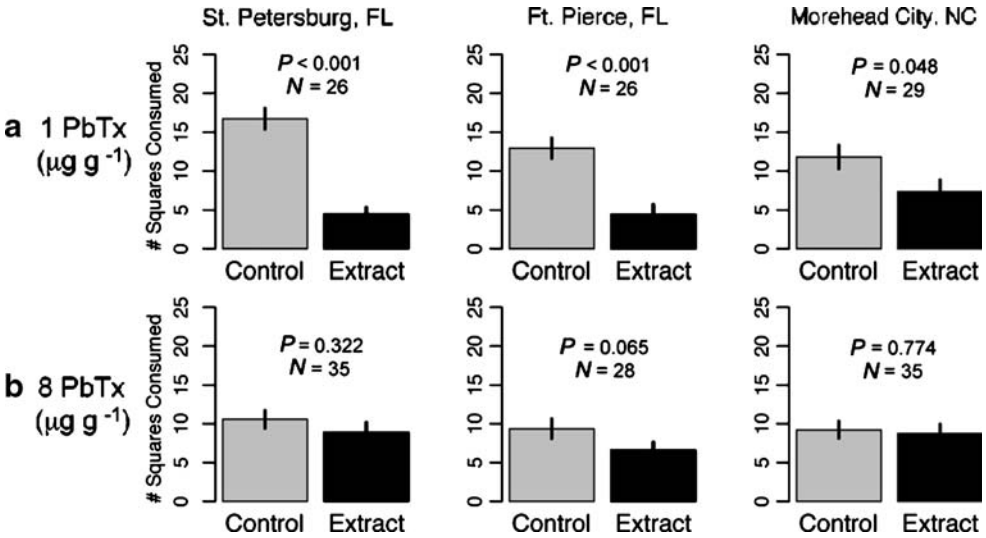

Fig. 2 Pairwise feeding choice by populations of the herbivorous amphipod *Ampithoe longimana*. Individuals were offered a feeding choice between freeze-dried *Ulva* (syn. *Enteromorpha*) *intestinalis* tissue coated with ether only (i.e., Control) or coated with *Karenia brevis* extracts at a concentration of **a**) one or **b**) eight μg brevetoxin (g^{-1} dry mass). Sample sizes and *P*-values from paired *t*-tests are indicated

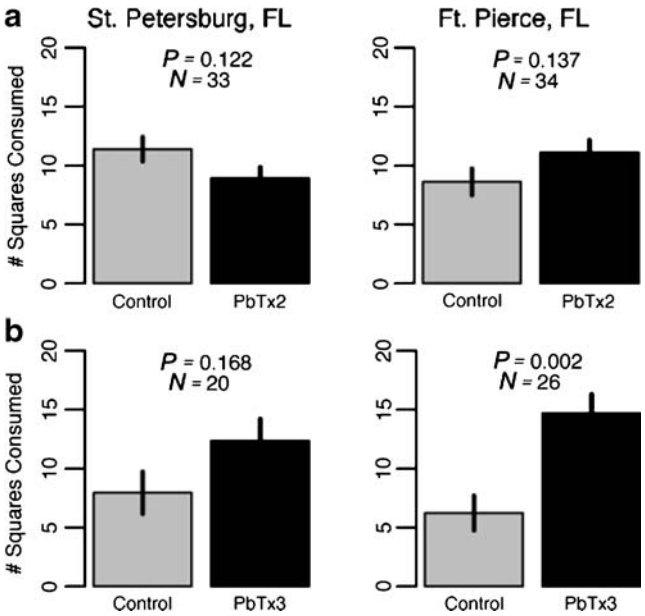

Fig. 3 Pairwise feeding choice by populations of the herbivorous amphipod *Ampithoe longimana*. Individuals were offered a feeding choice between freeze-dried *Ulva* (syn. *Enteromorpha*) *intestinalis* tissue coated with ether only (i.e., Control) or coated with **a**) PbTx2 or **b**) PbTx3 at a concentration of 1 μg brevetoxin (g^{-1} dry mass). Sample sizes and *P*-values from paired *t*-tests are indicated

herbivores, we offered a feeding choice between control and brevetoxin-coated foods at 1 μg g^{-1} to North Carolina populations of the sea urchin *Arbacia punctulata* and amphipod *Ampithoe valida.* Both herbivores readily consumed brevetoxin-coated foods at a rate equivalent to control foods (Fig. 5).

Discussion

As a first step toward understanding the potential importance of mesograzers in the vertical transmission of brevetoxins through benthic food webs of the Gulf of Mexico, we demonstrated that foods coated with observed 'post-bloom' concentrations of brevetoxins do not generally alter the feeding behaviors, survivorship, or growth of the benthic herbivorous amphipod *Ampithoe longimana* (Fig. 1–3, 4a). Moreover, adult amphipods revealed no decline in survivorship when exposed to solubilized brevetoxin at concentrations observed after blooms (Fig. 4b). We also found no detectable effects of *K. brevis* extracts on the feeding choices of two other benthic herbivores, the amphipods *A. valida* and purple sea urchin *Arbacia punctulata* (Fig. 5). Together, these results indicate that after *K. brevis* red tides, benthic herbivores readily feed on brevetoxin-coated foods, survive, and grow in brevetoxin-contaminated waters, and can, therefore, potentially transmit these brevetoxins to their predators.

Although the overall pattern is one of a lack of feeding deterrency, crude *K. brevis* extracts occasionally and inconsistently deterred amphipod feeding (see Results). Several lines of evidence suggest that this inconsistent feeding deterrence is mediated by unstable compounds eluted with 100% methanol extracts that are not brevetoxins. First, we found no change in total brevetoxin concentrations in the crude extracts over eight months of storage and handling (data not shown). Second, a direct test indicated that *A. longimana* was never deterred by purified brevetoxins at observed post-bloom concentrations (Fig. 3). In fact, the presence of PbTx-3 at 1 μg g^{-1} was a feeding attractant to a Fort Pierce population. Finally, we know that our methods were successful in offering brevetoxin-coated foods to the amphipods, because we detected brevetoxins within the tissues and/or guts of amphipods that were isolated on brevetoxin-laden foods for several weeks (see Results). The presence of unstable non-brevetoxin feeding deterrents similarly was inferred by Kubanek et al. (2007) when studying the feeding behaviors of rotifers.

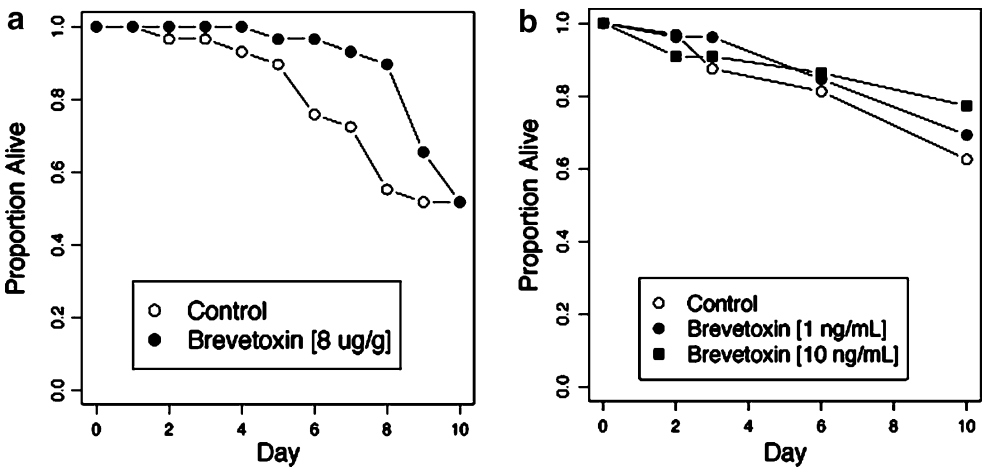

Fig. 4 Survivorship of Florida *Ampithoe longimana* when exposed to brevetoxins. **a**) Survivorship of juvenile amphipods when isolated on *Ulva* (syn. *Enteromorpha*) *intestinalis* tissue coated with ether only (i.e., Control) or *Karenia brevis* extracts at a concentration of eight μg brevetoxin (g^{-1} dry mass). **b**) Survivorship of amphipods when exposed to water with ethanol only (control), or observed (1 or 10 ng ml^{-1}) concentrations of purified brevetoxins

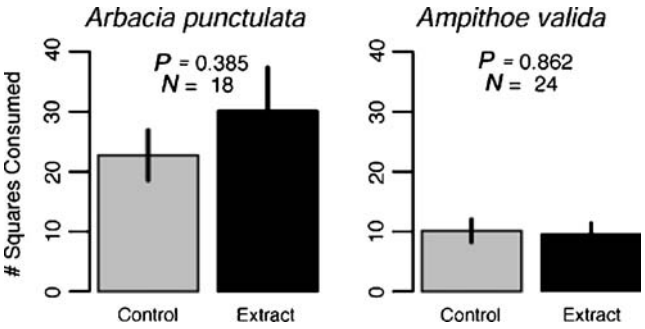

Fig. 5 Pairwise feeding choice by the temperate urchin *Arbacia punctulata* and the amphipod *Ampithoe valida*. Individuals were offered a feeding choice between freeze-dried *Ulva* (syn. *Enteromorpha*) *intestinalis* tissue coated with ether only (i.e., Control) or coated with *Karenia brevis* extracts at a concentration of one μg brevetoxin (g^{-1} dry mass). Sample sizes and *p*-values from paired t-tests are indicated

The lack of deterrent or deleterious effects of brevetoxins is surprising for several reasons. First, these results are inconsistent with observations of elevated mortality among benthic invertebrates when *K. brevis* blooms enter nearshore estuaries (e.g., Simon and Dauer 1972). However, it is difficult to assess whether our data and the mortality events truly are in conflict because brevetoxins loads were not measured during such events, and alternative or compounding explanations also could account for the catastrophic mortality event (e.g., hypoxia: Santos and Simon 1980). Second, the lack of a feeding deterrence seen in *A. longimana* appears to contrast strongly with the strongly deterrent effects of brevetoxins on other marine organisms. *Karenia brevis* cells and extracts appear to alter the behavior and fitness of pelagic rotifers (Kubanek et al. 2007) and copepods (Turner and Tester 1997; Landsberg et al. 2005; Prince et al. 2006; Breier and Buskey 2007; Cohen et al. 2007). In benthic habitats, *K. brevis* cells alter the feeding behavior, abundance and/or fitness of bivalves (Summerson and Peterson 1990; Keppler et al. 2006; Leverone et al. 2006; Haubois et al. 2007; Leverone et al. 2007). The causes of differences in feeding response toward *K. brevis* between groups of herbivores remain unclear, but we find it interesting that the previously studied herbivores consume live *K. brevis* cells, while the benthic herbivores studied here consume brevetoxin-laden epiphytes and seagrasses. Live *K. brevis* may occur occasionally on benthic plants, but unlike *Gambierdiscus* on tropical macroalgae (Cruz-Rivera and Villareal 2006), *K. brevis* does not sustain benthic populations.

We were also surprised that we could not detect significant differences in feeding behavior among amphipod populations that are annually exposed to *K. brevis* blooms (i.e., Tampa Bay; (Brand and Compton 2007) versus populations that rarely experience such blooms [i.e., Fort Pierce, Florida and Morehead City, NC; (Summerson and Peterson 1990)]. We had expected such microevolution to occur, because this amphipod has evolved population-level differences in feeding behaviors and fitness when isolated with lipophilic secondary metabolites produced by the brown seaweed *Dictyota* (Sotka and Hay 2002; Sotka et al. 2003; Sotka and Whalen 2008), and because some populations of a benthic clam (*Mya arenaria*; Bricelj et al. 2005) and pelagic copepod (*Acartia hudsonica*; Colin and Dam 2002) have evolved tolerance toward harmful algal blooms in the genus *Alexandrium*. It remains possible that population-level variation in *A. longimana* fitness traits is present (e.g., female fecundity) but this was not tested here.

A review by Landsberg (2002) concluded that the overall effects of harmful algal blooms (or HABs) "on food webs and ecosystems are probably the least understood of all impacts," and that "the pathways of algal-toxin transmission (are) complex and incompletely known" (pg. 188; see also Hay and Kubanek 2002; Cruz-Rivera and Villareal 2006). We propose that mesograzers and other benthic herbivores may represent an underexplored set of pathways by which brevetoxins produced by Gulf of Mexico *K. brevis* red tide blooms are transmitted vertically through estuarine food webs. Central to our hypothesis is the fact that mesograzers are a vitally important component of the diet for a variety of nearshore fishes (Motta et al. 1993; Luczkovich et al. 2002; Marancik and Hare 2007), including the extremely abundant pinfish *Lagodon rhomboides* (Stoner 1979). Moreover, the importance of mesograzers as a diet is greater for juvenile than adult life stages, suggesting that younger fish will be particularly vulnerable to brevetoxin transmission via mesograzer consumption. In support of our hypothesis, Naar et al. (2007) and Fire et al. (2008) document substantial loads of brevetoxins within *L. rhomboides* and other fishes in western Florida estuaries when collected weeks after a *K. brevis* blooms had relaxed.

We recognize that acceptance of benthic mesograzers as an additional mode of brevetoxin transmission requires answers to several outstanding questions. We do not know whether natural populations of mesograzers are laden with brevetoxins. We also do not know the rates at which bioaccumulation (if present) and depuration occurs in mesograzers. The brevetoxin load we report is much lower than that detected for pelagic copepods under laboratory conditions (~25 ng brevetoxin per copepod; Tester et al. 2000), although it is not clear whether these experiments (including the present study) utilized ecologically-realistic feeding rates. Moreover, we have not demonstrated that consumption of brevetoxin-laden mesograzers by fishes actually yields accumulation in fishes (e.g., Tester et al. 2000; Naar et al. 2007). Nevertheless, the present study does indicate that mesograzers deserve attention in our efforts to predict bioaccumulation rates and ecological impacts of brevetoxins within nearshore systems.

Acknowledgements We thank Marie DeLorenzo, Spencer Fire, Hannah Giddens, Tod Leighfield, and Zhihong Wang for brevetoxin analyses, fruitful discussion or both. N.O. was supported by an NSF Research Experience for Undergraduates fellowship (DBI-0552828) and the Department of Defense ASSURE Program. E.E.S. and A.M. were supported by a grant from NSF (OCE-0550245). This is Grice Marine Laboratory publication number 342 and NOAA CCEHBR CL-1460. The National Ocean Service does not approve, recommend or endorse any proprietary product or material mentioned in this publication.

References

ALCOCK, F. 2007. An assessment of Florida red tide: Causes, consequences and management strategies, p. 40. Technical Report #1190. Sarasota, FL: Mote Marine Laboratory.

BADEN, D. G. 1989. Brevetoxins: unique polyether dinoflagellate toxins. *FASEB J.* 3:1807–1817.

BADEN, D. G., BOURDELAIS, A. J., JACOCKS, H., and MICHELLIZA, S. 2005. Natural and derivative brevetoxins: historical background, multiplicity, and effects. *Environ. Health Perspect.* 113:621–625.

BRAND, L. E. and COMPTON, A. 2007. Long-term increase in *Karenia brevis* abundance along the Southwest Florida Coast. *Harmful Algae* 6:232–252.

BRAWLEY, S. H. 1992. Mesoherbivores, pp. 235–263, in D. M. John, S. J. Hawkins and J. H. Price (eds.). Plant-animal interactions in the marine benthos. Clarendon, Oxford.

BREIER, C. F. and BUSKEY, E. J. 2007. Effects of the red tide dinoflagellate, *Karenia brevis*, on grazing and fecundity in the copepod *Acartia tonsa*. *J. Plankton Res.* 29:115–126.

BRICELJ, V. M., CONNELL, L., KONOKI, K., MACQUARRIE, S. P., SCHEUER, T., CATTERALL, W. A., and TRAINER, V. L. 2005. Sodium channel mutation leading to saxitoxin resistance in clams increases risk of PSP. *Nature* 434:763–767.

BROOKS, R. A. and BELL, S. S. 2001. Mobile corridors in marine landscapes: enhancement of faunal exchange at seagrass/sand ecotones. *J. Exp. Mar. Biol. Ecol.* 264:67–84.

COHEN, J. H., TESTER, P. A., and FORWARD, R. B JR. 2007. Sublethal effects of the toxic dinoflagellate *Karenia brevis* on marine copepod behavior. *J. Plank. Res.* 29:301–315.

COLIN, S. P. and DAM, H. G. 2002. Latitudinal differentiation in the effects of the toxic dinoflagellate *Alexandrium* spp. on the feeding and reproduction of populations of the copepod *Arcatia hudsonica*. *Harmful Algae* 1:113–125.

CRUZ-RIVERA, E., and VILLAREAL, T. A. 2006. Macroalgal palatability and the flux of ciguatera toxins through marine food webs. *Harmful Algae* 5:497–525.

DARNELL, R. M. 1961. Trophic spectrum of an estuarine community, based on studies of Lake Pontchartrain, Louisiana. *Ecology* 42:553–568.

DOUCETTE, G., MANEIRO, I., RIVEIRO, I., and SVENSEN, C. 2005. Phycotoxin pathways in aquatic food webs: transfer, accumulation and degradation, pp. 285–295, *in* E. Graneliand and J. T. Turner eds. Ecology of Harmful Algae,. Springer-Verlag, Berlin.

FIRE, S. E., FLEWELLING, L. J., NAAR, J., TWINER, M. J., HENRY, M. S., PIERCE, R. H., GANNON, D. P., WANG, Z., DAVIDSON, L., and WELLS, R. S. 2008. Prevalence of brevetoxins in prey fish of bottlenose dolphins in Sarasota Bay, Florida. *Mar. Ecol. Prog. Ser.* 368:283–294.

FLEWELLING, L. J. 2008. Vectors of brevetoxins to marine mammals. Ph.D. dissertation. University of South Florida, 143 pp.

FLEWELLING, L. J., NAAR, J. P., ABBOTT, J. P., BADEN, D. G., BARROS, N. B., BOSSART, G. D., BOTTEIN, M. Y., 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., And LANDSBERG, J. H. 2005. Brevetoxicosis: red tides and marine mammal mortalities. *Nature* 435:755–756.

HAUBOIS, A. G., BRICELJ, V. M., and NAAR, J. P. 2007. Transfer of brevetoxins to a telliid bivalve by suspension-and deposit-feeding and its implications for clay mitigation of *Karenia brevis* blooms. *Mar. Biol.* 151:2003–2012.

HAY, M. E. and KUBANEK, J. 2002. Community and ecosystem level consequences of chemical cues in the plankton. *J. Chem. Ecol.* 28:2001–2016.

HUGHES, A., BANDO, K. J., RODRIGUEZ, L., and WILLIAMS, S. 2004. Relative effects of grazers and nutrients on seagrasses: a meta-analysis approach. *Mar. Ecol. Prog. Ser.* 282:87–99.

JERNAKOFF, P., BREARSLEY, A., and NIELSEN, J. 1996. Factors affecting grazer-epiphyte interactions in temperate seagrass meadows. *Oceanogr. Mar. Biol. Ann. Rev.* 34:109–362.

KEPPLER, C., LEWITUS, A., RINGWOOD, A., HOGUET, J., and STATON, T. 2006. Sublethal cellular effects of short-term raphidophyte and brevetoxin exposures on the eastern oyster *Crassostrea virginica*. *Mar. Biol. Prog. Ser.* 312:141–147.

KUBANEK, J., SNELL, T. W., and PIRKLE, C. 2007. Chemical defense of the red tide dinoflagellate *Karenia brevis* against rotifer grazing. *Limnol. Oceanogr.* 52:1026–1035.

LANDSBERG, J. 2002. The effects of harmful algal blooms on aquatic organisms. *Rev. Fish. Sci.* 10:113–390.

LANDSBERG, J., VAN DOLAH, F., and DOUCETTE, G. 2005. Marine and estuarine harmful algal blooms: impacts on human and animal health, pp. 165–215 *in* S. S. Belkin and R. Colwell, eds. Oceans and Health: Pathogens in the Marine Environment, Springer, New York.

LEVERONE, J. R., BLAKE, N. J., PIERCE, R. H., and SHUMWAY, S. E. 2006. Effects of the dinoflagellate *Karenia brevis* on larval development in three species of bivalve mollusc from Florida. *Toxicon* 48:75–84.

LEVERONE, J. R., SHUMWAY, S. E., and BLAKE, N. J. 2007. Comparative effects of the toxic dinoflagellate *Karenia brevis* on clearance rates in juveniles of four bivalve mollusks from Florida, USA. *Toxicon* 49:634–645.

LUCZKOVICH, J., WARD, G., JOHNSON, J., CHRISTIAN, R., BAIRD, D., NECKLES, H., and RIZZO, W. 2002. Determining the trophic guilds of fishes and macroinvertebrates in a seagrass food web. *Est. Coasts.* 25:1143–1163.

MARANCIK, K. E. and HARE, J. A. 2007. Large scale patterns in fish trophodynamics of estuarine and shelf habitats of the southeast United States. *Bull. Mar. Sci.* 80:67–91.

MAUCHER, J. M., BRIGGS, L., PODMORE, C., and RAMSDELL, J. S. 2007. Optimization of blood collection card method/enzyme-linked immunoassay for monitoring exposure of bottlenose dolphin to brevetoxin-producing red tides. *Environ. Sci. Tech.* 41:563–567.

MOTTA, P. J., CLIFTON, K. B., HERNANDEZ, P., and EGGOLD, B. T. 1993. Feeding relationships among nine species of seagrass fishes of Tampa Bay, Florida. *Bull. Mar. Sci.* 56:185–200.

NAAR, J. P., FLEWELLING, L. J., LENZI, A., ABBOTT, J. P., GRANHOLM, A., JACOCKS, H. M., GANNON, D., HENRY, M., PIERCE, R. H., BADEN, D. G., WOLNY, J., and LANDSBERG, J.H.. 2007. Brevetoxins, like ciguatoxins, are potent ichthyotoxic neurotoxins that accumulate in fish. *Toxicon* 50:707–723.

NELSON, W. G. 1980. A comparative study of amphipods in seagrasses from Florida to Nova Scotia. *Bull. Mar. Sci.* 30:80–89.

POLI, M., MUSSER, S., DICKEY, R., EILERS, P., and HALL, S. 2000. Neurotoxic shellfish poisoning and brevetoxin metabolites: a case study from Florida. *Toxicon* 38:981–993.

PRINCE, E. K., LETTIERI, L., MCCURDY, K. J. and KUBANEK, J. 2006. Fitness consequences for copepods feeding on a red tide dinoflagellate: deciphering the effects of nutritional value, toxicity and feeding behavior. *Oecologia* 147:479–488.

SANTOS, S. and SIMON, J. 1980. Response of soft-bottom benthos to annual catastrophic disturbance in a south Florida estuary. *Mar. Ecol. Prog. Ser.* 3:347–355.

SIMON, J. and DAUER, D. 1972. A quantitative evaluation of red-tide induced mass mortalities of benthic invertebrates in Tampa Bay. Florida. *Environ. Lett.* 3:229–234.

SOTKA, E. E. and WHALEN, K. 2008. Herbivore offense in the sea: the detoxification and transport of secondary metabolites, pp. 203–228 in C. Amsler (ed.). Algal chemical ecology, Springer-Verlag. Berlin.

SOTKA, E. E. and HAY, M. E. 2002. Geographic variation among herbivore populations in tolerance for a chemically-rich seaweed. *Ecology* 83:2721–2735.

SOTKA, E. E., WARES, J. P., and HAY, M. E. 2003. Geographic and genetic variation in feeding preference for chemically defended seaweeds. *Evolution* 57:2262–2276.

STEIDINGER, K. A., VARGO, G. A., TESTER, P. A., and TOMAS, C. R. 1998. Bloom dynamics and physiology of *Gymnodinium breve* with emphasis on the Gulf of Mexico, pp. 133–153 in D. M. Anderson, A. D. Cembella and G. M. Hallegraeff (eds.). Physiological ecology of harmful algal blooms, Springer-Verlag. Berlin.

STONER, A. 1979. Species-specific predation on amphipod crustacea by the pinfish *Lagodon rhomboides*: mediation by macrophyte standing crop. *Mar. Biol.* 55:201–207.

SUMMERSON, H., and PETERSON, C. 1990. Recruitment failure of the bay scallop, *Argopecten irradians concentricus*, during the first red tide, *Ptychodiscus brevis,* outbreak recorded in North Carolina. *Est. Coasts.* 13:322–331.

TESTER, P. A., SHEA, D., KIBLER, S. R., VARNAM, S. M., BLACK, M. D., and LITAKER, R. W. 2008. Relationships among water column toxins, cell abundance and chlorophyll concentrations during *Karenia brevis* blooms. *Cont. Shelf Res.* 28:59–72.

TESTER, P. A. and STEIDINGER, K. A. 1997. *Gymnodinium breve* red tide blooms: initiation, transport, and consequences of surface circulation. *Limnol. Oceanogr.* 42:1039–1051.

TESTER, P. A., TURNER, J. T., and SHEA, D. 2000. Vectorial transport of toxins from the dinoflagellate *Gymnodinium breve* through copepods to fish. *J. Plank. Res.* 22:47–61.

TURNER, J. T. and TESTER, P. A. 1997. Toxic marine phytoplankton, zooplankton grazers, and pelagic food webs. *Limnol. Oceanogr.* 42:1203–1214.

TWINER, M. J., DECHRAOUI, M. Y. B., WANG, Z., MIKULSKI, C. M., HENRY, M. S., PIERCE, R. H., and DOUCETTE, G. J. 2007. Extraction and analysis of lipophilic brevetoxins from the red tide dinoflagellate *Karenia brevis*. *Anal. Biochem.* 369:128–135.

VAN DOLAH, F., ROELKE, D., and GREENE, R. M. 2001. Health and ecological impacts of harmful algal blooms: risk assessment needs. *Hum. Ecol. Risk Assess.* 7:1329–1345.

VIRNSTEIN, R. W. 1987. Seagrass-associated invertebrate communities of the southeastern USA: a review, pp. 89–116 in M. J. Durako, R. C. Phillips and R. R. Lewis (eds.). Proceedings of the Symposium on subtropical-tropical seagrasses of the southeastern United States. Florida Marine Research Publication.

WANG, Z. H., PLAKAS, S. M., EL SAID, K. R., JESTER, E. L. E., GRANADE, H. R., and DICKEY, R. W. 2004. LC/MS analysis of brevetoxin metabolites in the Eastern oyster (*Crassostrea virginica*). *Toxicon* 43:455–465.