

Brevetoxin persistence in sediments and seagrass epiphytes of east Florida coastal waters

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ABSTRACT

A bloom of *Karenia brevis* Davis developed in September 2007 near Jacksonville, Florida and subsequently progressed south through east Florida coastal waters and the Atlantic Intracoastal Waterway (ICW). Maximum cell abundances exceeded 10^6 cells L^{-1} through October in the northern ICW between Jacksonville and the Indian River Lagoon. The bloom progressed further south during November, and terminated in December 2007 at densities of 10^4 cells L^{-1} in the ICW south of Jupiter Inlet, Florida. Brevetoxins were subsequently sampled in sediments and seagrass epiphytes in July and August 2008 in the ICW. Sediment brevetoxins occurred at concentrations of 11–15 ng PbTx-3 equivalents (g dry wt sediment) $^{-1}$ in three of five basins in the northern ICW during summer 2008. Seagrass beds occur south of the Mosquito Lagoon in the ICW. Brevetoxins were detected in six of the nine seagrass beds sampled between the Mosquito Lagoon and Jupiter Inlet at concentrations of 6–18 ng (g dry wt epiphytes) $^{-1}$. The highest brevetoxins concentrations were found in sediments near Patrick Air Force Base at 89 ng (g dry wt sediment) $^{-1}$. In general, brevetoxins occurred in either seagrass epiphytes or sediments. Blades of the resident seagrass species have a maximum life span of less than six months, so it is postulated that brevetoxins could be transferred between epibenthic communities of individual blades in seagrass beds. The occurrence of brevetoxins in east Florida coast sediments and seagrass epiphytes up to eight months after bloom termination supports observations from the Florida west coast that brevetoxins can persist in marine ecosystems in the absence of sustained blooms. Furthermore, our observations show that brevetoxins can persist in sediments where seagrass communities are absent.

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1. Introduction

The dinoflagellate *Karenia brevis* Davis (G. Hansen and Moestrup comb. Nov.) dominates harmful algal blooms (HABs) that develop on an almost annual basis in the eastern Gulf of Mexico (GOM). Dense aggregations of *K. brevis* co-occur with three other *Karenia* species (Steidinger et al., 2008) at cell densities of 10^5 – 10^6 cells L^{-1} in west Florida (WF) coastal waters. Blooms typically develop between Tampa and Ft. Myers, FL. *K. brevis* produces a suite of cyclic polyether neurotoxins known as brevetoxins (PbTx); the PbTx designation is derived from *Ptychodiscus brevis*, a prior epithet for *K. brevis* (Daugbjerg et al., 2000). The two primary brevetoxins synthesized within the cell are PbTx-1 and PbTx-2, and various analogs are derived from these ‘parent’ compounds (Baden et al., 2005). Florida ‘red tides’ frequently coincide with fish kills

and mortality events of marine reptiles, mammals and birds (Landsberg, 2002). Brevetoxins produced by *K. brevis* also impact human health when shellfish containing brevetoxins are consumed, producing Neurotoxic Shellfish Poisoning. Furthermore, brevetoxins can be aerosolized through wave action and produce respiratory irritation when inhaled (Fleming et al., 2005; Kirkpatrick et al., 2004). The overall impact of prolonged *K. brevis* blooms on Florida’s economy can approach \$20 million per year (Anderson et al., 2000).

Brevetoxins have been detected in marine organisms weeks to months after *K. brevis* blooms have terminated (Flewelling et al., 2005). Seagrass communities, for example, can retain brevetoxins at concentrations of several tens of ng (g dry wt) $^{-1}$ throughout the year in west Florida coastal waters, including periods when *K. brevis* is absent from the water column (Flewelling, 2008). In contrast to seagrass leaves, seagrass epiphytic communities were found to contain higher brevetoxins concentrations. Seagrass epiphyte communities are comprised of autotrophic (diatom, dinoflagellate, cyanobacteria) organisms as well as heterotrophs

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that range in size from bacteria to benthic filter-feeders such as bivalves. Planktivorous fish that ingest *K. brevis*, and benthic feeders that graze on seagrasses and their epiphytes, can retain brevetoxins in their tissues for weeks (Naar et al., 2007; Woofter et al., 2005). Individual brevetoxin analogs (PbTx-2 and PbTx-3) have been found in west Florida coastal sediments at concentrations of ca. 1–10 ng (g dry wt)⁻¹ when *K. brevis* is absent in the overlying waters (Flewelling, 2008; Mendoza et al., 2008). Collectively these studies indicate that brevetoxins persist for weeks to months in the coastal ecosystems of west Florida.

Although *K. brevis* blooms develop frequently along the west Florida coast, blooms also occur infrequently along the southeastern Atlantic coast (e.g., Murphey et al., 1975; Roberts, 1979). In 1987, a west Florida bloom was transported along the edge of the GOM Loop Current and the Florida Current-Gulf Stream system, and eventually developed as a 'red tide' off South Carolina and North Carolina. Similar blooms occurred in the South Atlantic Bight (SAB) in 1976, 1978, 1987 (Tester et al., 1991), and 1990 (Tester et al., 1993). A circulation model applied to the WF shelf indicates that winds, buoyancy, and the proximity to the GOM Loop Current are critical factors in the export of *K. brevis* from west Florida coastal waters (Weisberg et al., 2009).

In summer 2007 a bloom of *K. brevis* developed along the WF coast under conditions that favored the export of cells to the Loop Current with eventual transport to the SAB (Walsh et al., 2009). A subsequent *K. brevis* bloom was reported near Jacksonville, FL in mid-September 2007. By mid-October *K. brevis* had spread from St. Augustine south through the Intracoastal Waterway (ICW) and into the Indian River Lagoon (see Fig. 1). The Indian River Lagoon (IRL) is composed of inter-connected estuarine systems: the Mosquito Lagoon, The Banana River, which is within Cape Canaveral, and the Indian River. The Indian River is composed of an elongate basin inshore of barrier islands that connects to the Mosquito Lagoon on the north and terminates at St. Lucie Inlet. The Atlantic ICW extends through the IRL and continues along the east Florida (EF) coast. Fish kills were reported along the EF coast through December 2007 that were attributed to the presence of the ichthyotoxic *K. brevis* (Walsh et al., 2009).

Here we describe the relationship between the spatial distribution of the 2007 EF coastal bloom of *K. brevis* and the persistence of brevetoxins in sediment and seagrass epiphytes within the ICW in summer 2008. The southerly progression of the *K. brevis* bloom is described from weekly surveys conducted by the Florida Fish and Wildlife Conservation Commission. We sampled brevetoxins in the sediments and seagrass epiphyte communities in the ICW in July–August, 2008 between Jacksonville, FL and Jupiter Inlet, FL, a distance of ca. 400 km.

2. Materials and methods

2.1. *K. brevis* abundance

Samples are collected weekly from Florida coastal waters for the HAB monitoring program of the Florida Fish and Wildlife Conservation Commission (FWC) (Heil and Steidinger, 2009). Surface waters are sampled each week by local, State, and Federal government agencies, as well as academic institutions and nongovernmental organizations. Water samples are preserved with Lugol's solution and sent to the Florida Wildlife Research Institute in St. Petersburg, FL. The species identification and enumeration of HAB species is conducted by microscopic examination.

Counts of *K. brevis* and congeners are disseminated weekly on the World Wide Web, and the data is archived in the Florida HAB Historical Database. Current and archived data are available at <http://research.myfwc.com/features/default.asp?id=1018>. Al-

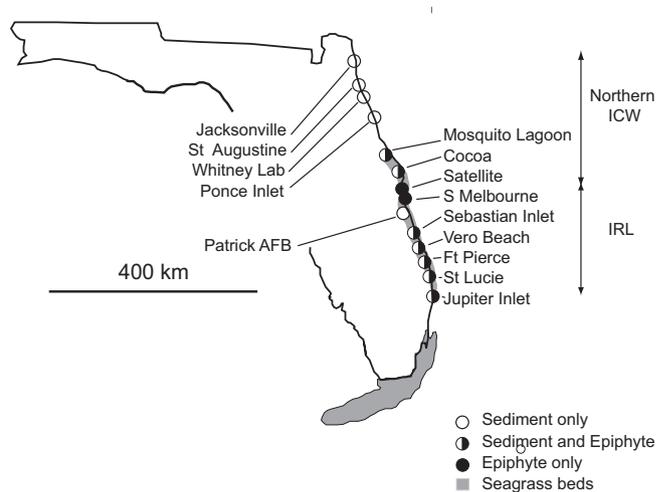


Fig. 1. Map of the Florida east coast. The Intracoastal Waterway (ICW) extends along the Florida coastline inshore of barrier islands. The northern ICW is designated as the portion from the state border, near Jacksonville FL (J), to the southern end of the Mosquito Lagoon (ML). St. Augustine (StA) and Ponce Inlet (P) are within the Northern ICW. The Indian River Lagoon connects to the Mosquito Lagoon at its northern end and extends south to St. Lucie Inlet. The Banana River (BR) and Patrick Air Force Base (PAF) are on Cape Canaveral. The IRL extends south of the Cape to Sebastian Inlet (Seb), Ft. Pierce Inlet (FtP), St. Lucie Inlet (StL) and Jupiter Inlet (Jup).

though samples are sporadically collected from east Florida coastal waters, an intensive sampling program was conducted during summer 2007 to monitor the bloom distribution. Surface samples were collected weekly at more than 30 locations in the ICW and EF beaches between September and December. Sampling has continued in EF coastal waters since the termination of the 2007 bloom, although on a reduced scale.

2.2. Sediment sampling

Sediment samples were collected from 17 sites on the EF coast in July and August 2008 (Table 1). Weather events, such as thunderstorms, dictated when and where samples could be collected at individual sites with a small boat. Surface sediments were collected by hand after wading into shallow waters. Care was taken not to disturb sediments before sampling. Material was taken from the upper 5 cm with a 2.5-cm diameter open-end plastic syringe that was capped as it was pulled from the sediments. Sediments were extruded from the syringe into a solvent-cleaned glass jar, capped, placed on ice for transport, and frozen at the laboratory at the University of Miami. The contents of the jars were transported on dry ice to the laboratory at Wilmington, NC for analysis following the protocol of Mendoza et al. (2008).

After the sediments were lyophilized and dry weights obtained, the brevetoxins were extracted with a solvent of 1:1 dichloromethane:acetone and sonication. An internal standard of PbTx-9 was added to the dried sample before the extraction procedure. Following centrifugation the solvent layer was transferred to a glass vial, and the process repeated three more times. The pooled sample was dried under high purity N₂ and held at -20° C. The dried material was suspended in 5 ml of a 85:15 (v/v) mixture of methanol:water with sonication for HPLC analysis.

The LC–MS analyses conditions were modified after Cheng et al. (2005). The extracts were separated by an Agilent 1100 LC series using a Phenomenex, LUNA, 3 μm, 50 mm × 2.0 mm, C18 column. The mobile phase was 50:50 acetonitrile:water (0.3% acetic acid) for the first 40 min, then changed to 5:95 acetonitrile:water for 2 min and finally back to 50:50 acetonitrile:water for 8 min. The flow rate was set at 0.2 ml min⁻¹.

Table 1

Sample location with latitude, longitude, date and local time (EDT) of collection for sediment and seagrass epiphyte materials assayed for brevetoxin concentration in east Florida coastal waters, summer 2008. The samples are listed by latitude.

Location	Latitude	Longitude	Date	Time
Jacksonville	30.3699	81.3966	July 23	0945
St. Augustine	29.7682	81.2579	July 22	1825
Whitney ML	29.6702	81.2160	July 22	1746
Ponce Inlet	29.0735	80.9169	July 23	1520
New Smyrna Beach	29.0715	80.9104	July 23	1552
Mosquito Lagoon*	28.8090	80.7817	August 5	1220
Cocoa Beach	28.3572	80.6286	August 5	1420
Patrick AFB	28.2331	80.5996	July 22	1422
Satellite Beach*	28.1781	80.5897	July 22	1406
Melbourne	28.0700	80.5790	August 5	1730
S. Mello Beach	27.9560	80.5009	July 22	1328
South Melbourne*	27.9520	80.5163	August 6	1130
Sebastian*	27.7890	80.4400	August 6	1315
Vero Beach*	27.6840	80.3860	August 6	1430
Ft. Pierce*	27.4820	80.3330	August 6	1700
St. Lucie Inlet*	27.1793	80.1818	August 7	0845
Jupiter Inlet*	26.9740	80.0831	August 6	1130

* Site where epiphytes were collected.

Structural identification, detection and quantification was carried out using a Thermo Finnigan TSQ Quantum triple quadrupole mass spectrometer, equipped with the electrospray ionization source (Thermo Fisher Scientific, Inc., USA). Nitrogen gas was used as both drying gas and nebulizing gas. The instrument was run using the selective reaction monitoring (SRM) mode. Optimized collision induced decomposition MS/MS spectra for each of the PbTx transitions were acquired at 4 V (PbTx-2), 18 V (PbTx-3) using argon as the collision gas at a pressure of 1.0 mTorr.

The SRM transitions of the two types of toxins were set at 895.5–877.5 m/z (PbTx-2) and 897.5–725.3 m/z (PbTx-3). Scan events of product ions were set at scan width of 1.0 s with scan time of 0.25 s. Q1 and Q3 peak width were set at 0.70 s. Total acquired segment time ran for 40 min. The HPLC–MS/MS limit of detection was similar to Cheng et al. (2005) at 2.5 ng ml⁻¹.

2.3. Seagrass epiphyte samples

Seagrass epiphytes were present at a sufficient density to provide samples at nine locations (Table 1). The epiphyte samples were collected concurrently with sediment samples. Shoots of *Syringodium filiforme* and *Halodule wrightii* were gently harvested by hand from depths of <1 m, placed in plastic bags, and stored on ice for transport to shore. In WF coastal ecosystems *Thalassia testudinum* is often the most abundant dominant seagrass, with *H. wrightii* and *S. filiforme* comprising a lower proportion of the seagrass community. *testudinum* is much less abundant in the EF coastal ecosystems we sampled than in the WF coastal ecosystems.

Epiphytes were harvested within eight hours of collection by scraping the surface community from each shoot with a clean razor blade. The scraped material was collected on waterproof paper and transferred to a cleaned glass test tube with a Teflon-lined cap. Samples were frozen for transport to the lab. The epiphyte material was lyophilized and dry weights determined prior to a triple extraction with HPLC-grade methanol. The combined extracts were dried under N₂ gas in solvent-cleaned test tubes. Each composite sample was then assayed for PbTx-3 equivalent concentrations in the laboratory of Dr. Kathleen Rein at Florida International University using the competitive enzyme-linked immunosorbent assay (ELISA) procedure of Naar et al. (2002).

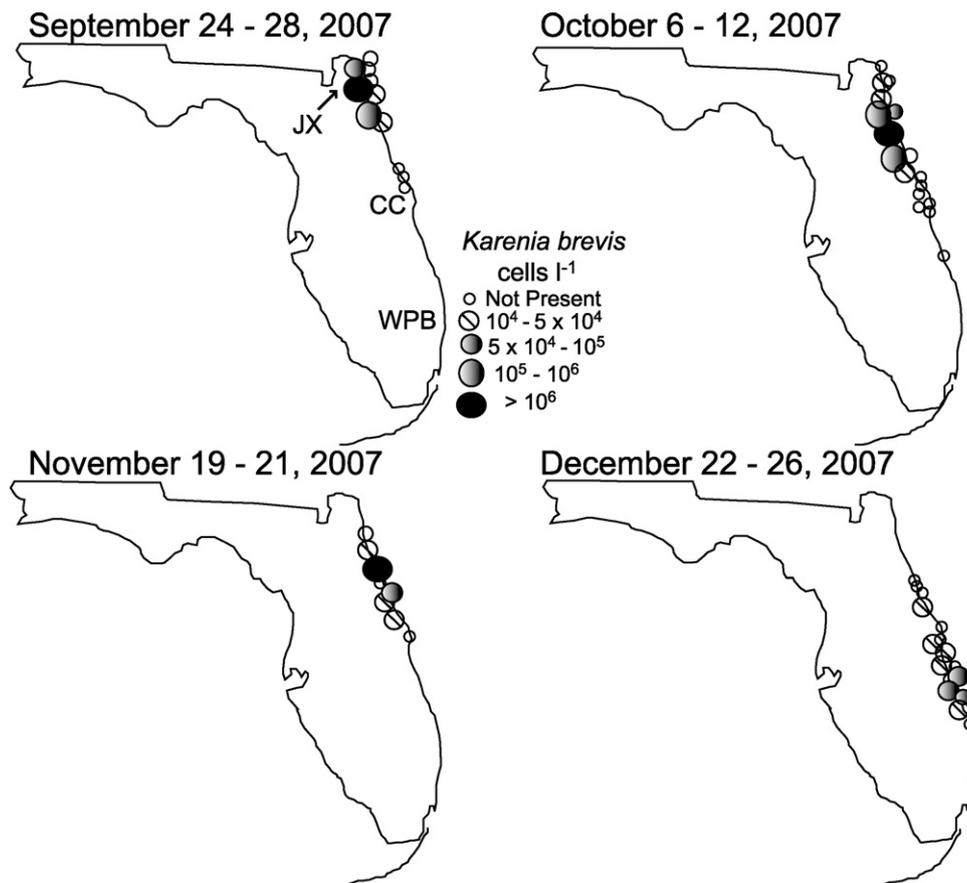


Fig. 2. The distribution of *Karenia brevis* along the east Florida coast in 2007.

Source: FWRI HAB Monitoring Program. The location of Jacksonville (JX), Cape Canaveral (CC) and West Palm Beach (WPB) are provided for reference.

The ELISA method provides a 'total' equivalent normalized to PbTx-3 (Naar et al., 2002). The competitive ELISA method has been successfully applied to quantify brevetoxin tissue burdens in shellfish (Naar et al., 2002), marine mammals (Flewelling et al., 2005), and fish (Fire et al., 2008; Naar et al., 2007), as well as seagrass and epiphytes (Flewelling et al., 2005; Flewelling, 2008), seawater, and marine aerosols (Pierce et al., 2005). Antibodies for the assay were derived from goats immunized with PbTx-3 conjugates (see Naar et al., 2007; Trainer and Baden, 1991). Although the method does not permit quantification of individual brevetoxins and their analogs, there is agreement between ELISA and HPLC–MS determined brevetoxins quantities in tissue samples from shellfish (Plakas et al., 2004; Pierce et al., 2006).

3. Results

3.1. The 2007 *K. brevis* bloom

The 2007 bloom of *K. brevis* along the east coast of Florida was first observed in early September near Jacksonville, FL (Walsh et al., 2009). Thereafter, the bloom expanded to the south as summarized by the east coast distribution from the FWC HAB monitoring program (Fig. 2). There were relatively few stations with detectable *K. brevis* in the first three weeks of September. In the last week of September, a bloom was evident in the ICW at Hanna Park, near Jacksonville, FL where cell abundances exceeded 10^6 L^{-1} (Fig. 2). Following the initial detection of the bloom, the highest densities were found progressively south throughout October and November. By mid-October maximum densities along the east coast were recorded near St. Augustine (Figs. 1 and 2). Maximum cell concentrations exceeding $10^6 \text{ cells L}^{-1}$ occurred in the ICW throughout October. While *K. brevis* was detectable along the beaches, the highest concentrations were in samples collected from the ICW.

In late November *Karenia* abundance still exceeded $10^6 \text{ cells L}^{-1}$ near Cape Canaveral with $>5 \times 10^6 \text{ cells L}^{-1}$ adjacent to Patrick Air Force Base. Cell densities remained at $>10^6 \text{ cells L}^{-1}$ in the first week of December in the Indian River Lagoon, Banana River, and ICW adjacent to Cape Canaveral. By mid-December the maximum cell concentrations in the southern ICW were 10^5 – $10^6 \text{ cells L}^{-1}$ (Fig. 2). By late December, the maximum cell concentrations detected in EF coastal waters were $3 \times 10^5 \text{ cells L}^{-1}$, with highest concentrations between St. Lucie and Jupiter Inlet. Maximum abundances recorded at the southern terminus of the 2007 bloom were $10^3 \text{ cells L}^{-1}$ at Jupiter Inlet FL. By early January 2008 *K. brevis* was nearly absent from the ICW, with isolated patches present at cell abundances of only 10^2 – $10^3 \text{ cells L}^{-1}$ (data not shown). No *K. brevis* was found in the ICW after February 2008.

3.2. Sediment brevetoxin distribution

Detectable brevetoxins concentrations were present in five of the sediment samples collected in July and August 2008, although the distributions were spatially heterogeneous (Fig. 3). The northernmost station at Hanna Park, near Jacksonville, FL, had water column cell concentrations exceeding $10^6 \text{ cells L}^{-1}$ in September 2007 with no detectable sediment brevetoxins in July 2008. *K. brevis* cell concentrations also exceeded $10^6 \text{ cells L}^{-1}$ in water samples collected in July 2007 between St. Augustine and the Mosquito Lagoon. However, sediment brevetoxin concentrations during the following summer were $1.3 \text{ ng (g dry wt)}^{-1}$ and $11.4 \text{ ng (g dry wt)}^{-1}$ at St. Augustine and the Whitney Laboratory, respectively. Further south, brevetoxins were absent from sediment samples at Ponce Inlet, but were present at $18.1 \text{ ng (g dry wt)}^{-1}$ in the Mosquito Lagoon.

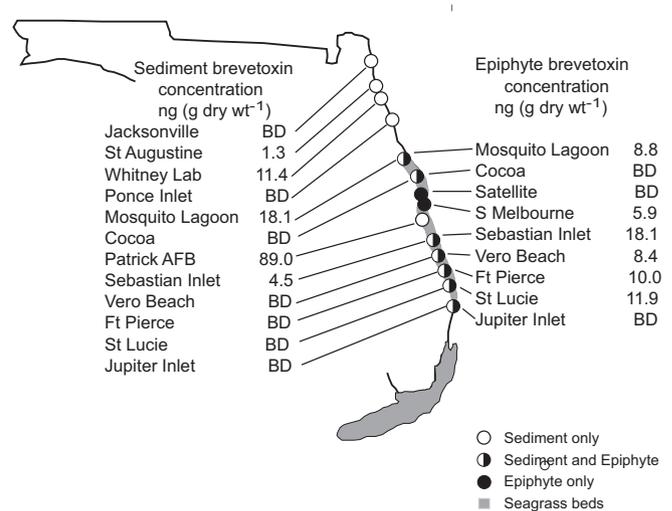


Fig. 3. Brevetoxin concentrations in sediment and seagrass epiphytes collected during July and August 2008 along the east Florida coast. Concentrations are expressed as ng PbTx-3 equivalents (g dry wt)⁻¹ of sample for sediments (left) and seagrass epiphytes (right). BD = below detection limit.

The highest sediment concentrations along the EF coast occurred in the IRL near Patrick Air Force Base (Fig. 3). This location corresponded to the October 2007 bloom epicenter (Fig. 2). The LC–MS analyses revealed that Patrick Air Force Base was the only location at which both parent brevetoxins were present. This sediment sample contained detectable concentrations of PbTx-1 at $0.9 \text{ ng (g dry wt)}^{-1}$, a parent brevetoxin with the A backbone (Baden et al., 2005). A second parent brevetoxin, PbTx-2 with the B backbone, was also present at $15.0 \text{ ng (g dry wt)}^{-1}$. Three additional brevetoxins found at this location were PbTx-3 ($89.0 \text{ ng g dry wt}^{-1}$), PbTx-6 ($1.0 \text{ ng g dry wt}^{-1}$) and PbTx-9 ($4 \text{ ng g dry wt}^{-1}$), which are all analogs of PbTx-2. Much lower brevetoxin concentrations were found further south at Sebastian Inlet, where there was only $4.5 \text{ ng (g dry wt)}^{-1}$. No brevetoxins were detected further south in the IRL sediments.

3.3. Seagrass epiphyte brevetoxin distribution

In general, epiphyte brevetoxins were not uniformly distributed in the ICW. Concentrations ranged from non-detectable to a maximum of $18 \text{ ng PbTx-3 (g dry wt)}^{-1}$. In the northernmost sample from the Mosquito Lagoon brevetoxin was present at $8.8 \text{ ng (g dry wt)}^{-1}$. In the vicinity of Cape Canaveral, seagrass epiphytes at Cocoa Beach and Satellite Beach had no detectable brevetoxins, while epiphyte communities sampled at South Melbourne contained $0.9 \text{ ng (g dry wt)}^{-1}$.

The five seagrass beds sampled in the southern ICW contained detectable epiphyte brevetoxins at four of the five stations. These beds contain higher seagrass abundances than those located further north (Morris et al., 2000). Concentrations varied from 8.4 to $18.1 \text{ ng (g dry wt)}^{-1}$ in four seagrass beds sampled between Sebastian Inlet and St. Lucie Inlet where sediment brevetoxins were absent (Fig. 3). The southernmost sample collected at Jupiter Inlet had no detectable brevetoxin in either the seagrass epiphytes or the sediments.

4. Discussion

4.1. Sediment brevetoxin distributions

Concentrations of sediment brevetoxins along the EF coast ranged from below detection to a maximum of $89 \text{ ng (g dry wt)}^{-1}$,

with most common values <20 ng (g dry wt) $^{-1}$. These concentrations are comparable to, but generally less than, those in WF coastal sediments during non-bloom periods. A sediment survey utilizing ELISA analyses was completed in sediments adjacent to Charlotte Harbor in March through August 2002, and found concentrations from non-detectable to 62 ng (g dry wt) $^{-1}$ with most values at 12–17 ng (g dry wt) $^{-1}$ during non-bloom periods (Flewelling, 2008). Values exceeded 1000 ng (g dry wt) $^{-1}$ during *K. brevis* blooms in 2002 (Flewelling, 2008). A subsequent study in April–December 2005 found 60–170 ng (g dry wt) $^{-1}$ in sediments following the decline of a dense *K. brevis* bloom. In regions where the dinoflagellate was not present, sediment concentrations were undetectable to 43 ng (g dry wt) $^{-1}$. Further south, near Ft. Myers, Mendoza et al. (2008) reported <1 –12 ng (g dry wt) $^{-1}$ in sediments from three locations in December 2006. As in our study, Mendoza and colleagues utilized liquid chromatography–mass spectrometry analyses to identify analogs. They reported 0.8–3.6 ng PbTx-2 (g dry wt) $^{-1}$ at two stations, and 2.7–9.7 ng PbTx-3 (g dry wt) $^{-1}$. Differences in analog concentrations in the west Florida sediments were attributed to the relative energy environment of sedimentary environments near Passes and at beaches.

Along the EF coast, high sediment brevetoxin occurred in the interior IRL (Patrick Air Force Base), and the Mosquito Lagoon. The highest sediment brevetoxins in the ICW occurred at Patrick Air Force Base where high cell concentrations (10^5 – 10^6 cells L $^{-1}$) persisted in the IRL for more than a month in 2007. The presence of additional analogs in sediments at Patrick Air Force Base suggests that forms other than PbTx-2 and PbTx-3 could persist under favorable conditions in addition to PbTx-2 and PbTx-3. Brevetoxins were not detected in the sediments near inlets with extensive seagrass beds in the southern IRL. Our results support the hypothesis posed by Mendoza that less energetic conditions in lagoons may enhance deposition and the long-term persistence of brevetoxins, relative to the higher energy environment found near inlets.

4.2. Seagrass epiphyte communities

Epiphyte brevetoxin concentrations of 6–18 ng (g dry wt) $^{-1}$ primarily occurred in seagrass beds in the southern IRL. These seagrass beds are densest at inlets that connect the IRL to the Atlantic Ocean (Morris et al., 2000). On the WF coast, seagrass epiphyte concentrations in the 2002 and 2005 studies were generally <50 ng (g dry wt) $^{-1}$ during periods when *K. brevis* was present at $<10^3$ cells L $^{-1}$ (Flewelling, 2008). During blooms, in contrast, epiphyte concentrations attain values as high as 1000–3000 ng (g dry wt) $^{-1}$ in 2002 and 2005, respectively (Flewelling, 2008). Thus, brevetoxin concentrations in the east Florida seagrass epiphyte communities were within the range, though generally lower, than that in WF seagrass beds during non-bloom conditions.

Seagrass species composition could have influenced epiphyte brevetoxin concentrations in our samples from EF coastal populations, where *H. wrightii* and *S. filiforme* are the most abundant species. On the WF coast, in contrast, seagrass beds frequently have *T. testudinum* as the most abundant species, with *H. wrightii* and *S. filiforme* present at lower abundances. Brevetoxin concentrations are higher per unit dry weight in *T. testudinum* epiphyte communities than in other seagrasses, and brevetoxin may persist in *T. testudinum* for longer periods following bloom termination (Flewelling, 2008). The differences were attributed to morphology of *Thalassia* blades, which are wider, and could potentially provide enhanced retention of detritus.

Brevetoxin persistence in seagrass epiphytes for periods of several months following bloom termination suggests that some vector or processes transfers toxin between individual leaves. The average age of leaves for *T. testudinum* (Patriquin, 1973; Tomasko

et al., 1996) and *H. wrightii* (Morgan and Kitting, 1984) are on the order of 30–40 days. *S. filiforme* blades have a comparable life span at 50 days (Zieman et al., 1979). The maximum life span of seagrass leaves of *H. wrightii* and *S. filiforme* is less than 50 days, while that of *T. testudinum* is <100 days (see Fig. 1, Borowitzka et al., 2006).

The presence of brevetoxins six months after bloom termination in the ICW indicates that the toxins must be transferred within the seagrass bed as individual blades die, as supported by observations from west Florida coastal ecosystems (Flewelling, 2008). Motile organisms are likely one potential mechanism for brevetoxin transfer between individual seagrass blades. Motile invertebrate mesograzers such as copepods, isopods, and amphipods live several months and are primary grazers of seagrass epiphytes (Jernakoff et al., 1996). Amphipods are relatively resistant to brevetoxin toxicity (Sotka et al., 2009) and could likely serve as a vector transferring toxins in seagrass communities. In addition, some mobile filter feeders like the wing oyster *Pinctada imbricata*, can detach from senescing seagrass blades and reattach to living blades in the canopy (J. Fourqurean, personal observations), providing another potential vector.

4.3. Conclusions

The persistence of brevetoxins in sediments and epiphytes has led to the hypothesis that brevetoxins could serve as markers for prior blooms (Flewelling, 2008; Mendoza et al., 2008). The processes mediating brevetoxin transfer from *K. brevis* to sediments and seagrass epiphyte communities is largely unknown, as are *in situ* rates of toxin degradation. While routes of brevetoxin transfer have been documented in experimental pelagic communities (Turner and Tester, 1997; Tester et al., 2000) there are, to our knowledge, no comparable studies of toxin transfer in sediments or seagrass epiphyte communities.

One process that may contribute to toxin transfer to benthic communities is swimming behavior of bloom organisms. *K. brevis* frequently undergoes a diurnal vertical migration, as cells frequently swim descend from the surface near dusk (e.g., Heil, 1986). In an experiment, cultured *K. brevis* has been observed to swim into the interstitial space between sand grains (Sinclair and Kamykowski, 2006). This behavior could provide a vector for transfer of brevetoxins to sediments. Additionally, *K. brevis* has been reported to form a cyst in culture (Walker, 1982), with an additional report of a palmelloid stage in culture and in sediments from the WF shelf (Steidinger et al., 1998). While the full life history of this species has yet to be fully documented, the collective observations suggest that behavior and the life cycle could contribute to the transfer of brevetoxins into benthic communities. Experimental studies with cultures and mesocosms should address these potential routes of transfer, and further validate the use of sediment and epiphyte brevetoxins as biomarkers for this harmful algal species.

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