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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 Moestrupcmb. 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. Although 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.

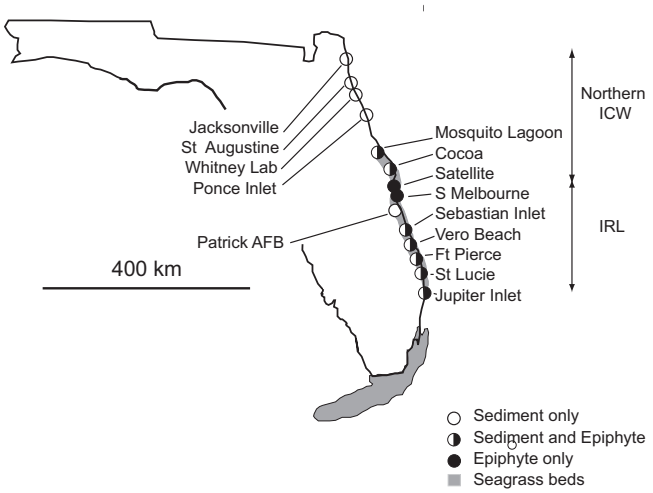

Fig. 1. Map of the Florida east coast. The Intracoastal Waterway (ICW) extends along the Florida coastline inshore of barrier islands. The northern ICW is designated as the portion from the state border, near Jacksonville FL (J), to the southern end of the Mosquito Lagoon (ML). St. Augustine (StA) and 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).

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 N_2 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}.

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