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Inland Transport of Aerosolized Florida Red Tide Toxins

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Abstract

Florida red tides, an annual event off the west coast of Florida, are caused by the toxic dinoflagellate, *Karenia brevis*. *K. brevis* produces a suite of potent neurotoxins, brevetoxins, which kill fish, sea birds, and marine mammals, as well as sickening humans who consume contaminated shellfish. These toxins become part of the marine aerosol, and can also be inhaled by humans and other animals. Recent studies have demonstrated a significant increase in symptoms and decrease lung function in asthmatics after only one hour of beach exposure during an onshore Florida red tide bloom.

This study constructed a transect line placing high volume air samplers to measure brevetoxins at sites beginning at the beach, moving approximately 6.4 km inland. One non-exposure and 2 exposure studies, each of 5 days duration, were conducted. No toxins were measured in the air during the non-exposure period. During the 2 exposure periods, the amount of brevetoxins varied considerably by site and by date. Nevertheless, brevetoxins were measured at least 4.2 kilometers from the beach and/or 1.6 km from the coastal shoreline. Therefore, populations sensitive to brevetoxins (such as asthmatics) need to know that leaving the beach may not discontinue their environmental exposure to brevetoxin aerosols.

Keywords

brevetoxins; harmful algal blooms (HABs); red tides; *Karenia brevis*; asthma; air monitoring

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1.0 Introduction

Florida red tides are an annual event off the West Coast of Florida and throughout the Gulf of Mexico, caused by blooms of the toxic dinoflagellate, *Karenia brevis* (*K. brevis*). *K. brevis* produces a suite of potent neurotoxins, brevetoxins, leading to significant fish, sea bird, and marine mammal mortality. In addition, these toxins are filtered and accumulated by bivalves such as clams and oysters; people who consume contaminated shellfish become ill with neurotoxic shellfish poisoning (NSP) (Baden et al., 1995; Keynes, 1979; Kirkpatrick et al., 2004; Watkins et al., 2008). *K. brevis* is an unarmored dinoflagellate that can be easily lysed, releasing brevetoxins into the water. Therefore, *K. brevis* brevetoxins can be incorporated into the marine aerosol (Pierce et al., 1990). When these toxins are inhaled, brevetoxins can cause upper and lower airway symptoms, particularly in people with chronic respiratory illnesses such as asthma (Fleming, et al., 2005; Fleming et al., 2007; Fleming et al., 2009). For many years, the public health message has been that symptoms of exposure to aerosolized Florida red tide would diminish when people left the beach (Steidinger and Baden, 1984; Baden 1983). However, anecdotal reporting from the community during onshore blooms have suggested that this may not be true for all people, particularly those with underlying respiratory diseases (Kirkpatrick et al., 2006, Kirkpatrick et al., 2009). Therefore, both exposure and health effects from brevetoxins in marine aerosols need to be measured not only at the beach, but also inland from the beach.

Red tide aerosols are marine aerosols containing aerosolized brevetoxins produced in the air/water interface and they are transported by onshore wind to coastal areas (Cheng et al., 2005; Pierce et al., 1990). Pierce and Cheng have reported on the methods to collect and quantify the amount of airborne toxins at the beach during an onshore Florida red tide (Cheng et al., 2005; Pierce et al., 2005). In our previous beach studies, brevetoxin levels measured in the air have ranged from 1.32 ng/m³ (Cheng et al., 2005) to as high as 93 ng/m³ (Backer et al., 2003). This study explored whether brevetoxin exposure continues beyond the beach, and if so, how far inland the brevetoxins travel.

2.0 Methods

This study is part of a larger research program investigating the exposures and health effects in humans and animals from aerosolized brevetoxins (Cheng et al., 2005; Fleming, et al 2005a; Fleming, 2005b; Fleming et al., 2007; Fleming et al., 2009; Pierce et al., 2005). In these “beach studies,” environmental sampling was performed during a 3 day period prior to the beginning of this “transect study” at the beach (Figure 1, site A), and then continued throughout the transect study described below. The environmental sampling was performed to establish the presence or absence of a *Karenia* bloom (via *K. brevis* cell count in the water, and brevetoxin levels in the water and air samples) as described in prior study publications (Cheng et al., 2005, Pierce et al., 2005).

2.1 Sampler Locations and Sample Collection

An inland transect line for the placement of the environmental air samplers was established beginning at Siesta Key Beach (Florida) (Site A), moving eastward/inland to a point approximately 6.4 km from the beach study site (Site F). Six high volume samplers (TE-5000, Tisch Environmental Inc., Village of Cleaves, OH, USA) were placed in locations of convenience to provide electrical power for the samplers and ease of changing filters daily (Figure 1). The samplers were fitted with 20 cm × 28 cm glass fiber filters (Whatman EPM 2000, Maidstone, England). Air samples were collected continuously from approximately 8 am to 5 pm daily for a period of 5 days.

2.2 Toxin Analysis

As described in Pierce, et al., (2005), brevetoxins from the marine aerosol were recovered from the glass fiber filters by extraction for 12 h in acetone using a Soxhlet apparatus. The extract was evaporated and transferred to vials for liquid chromatography mass spectroscopy (LC-MS) analysis. Brevetoxin recovery was verified by the addition of standard amounts of brevetoxin PbTx-2 and PbTx-3 to each of the three filters, the filters were run for 4 hours, and subsequently processed for LC-MS analysis.

Brevetoxin analyses were performed by LC-MS using a ThermoFinnigan AqA HPLC/MS obtained from Thermo Electron Corp., Manchester, United Kingdom. The LC consisted of a SpectraSystems, LC Pump P4000, Autosampler AS3000, and a Degasser SCM1000. Mass spectral detection was obtained using an AqA single quad system scanned from 204–1216 AMU with AqA Max 40, and a scan rate of 1.1 scans/second. All analysis was conducted using electrospray with the probe at 3 kV and 250°C. The column was a Phenomenex Security Guard C-18 guard column with a Phenomenex Luna C-18 5Fm 250 mm × 2 mm Analytical Column. The solvent gradient was composed of acidified (0.3% acetic acid) ACN/H²O over 40 minutes. The instrument was calibrated with a standard brevetoxin mix containing PbTx-2 and PbTx-3, obtained from the Center for Marine Science, UNC Wilmington, NC, USA.

3.0 Results

Table 1 lists the environmental conditions and water and air brevetoxin measurements at Site A during the beach studies prior to the 5-day transect studies. Table 2 lists the environmental conditions and air concentration of brevetoxins during the transect studies.

3.1 Non exposure (control) beach studies

Non-exposure beach studies were defined when no *K. brevis* cells were measured above detectable levels (<1,000 cells/L) in the beach waters at Site A (see Figure 1) and were conducted from October 16–18, 2004. Additionally, no brevetoxins were detected in the water or aerosol samples above the limits of detection by LC-MS (<0.05 ng/m³ air, 0.03 µg/L water) as shown in Table 1.

3.2 Exposure beach studies

Exposure beach studies were defined as *K. brevis* red tide events (cell counts >1,000 cells/L), and were conducted from February 4–6, 2005 and March 11–14, 2005. A summary of the cell counts from water samples and the total brevetoxins detected in water and aerosol samples for the beach studies along with *K. brevis* cell counts is presented in Table 1. In the beach study of February 4–6, 2005, the cell counts were above 1×10⁶/L, and the brevetoxin concentrations in the water were also high in the range of 13–128 µg/L. However, the air concentrations of brevetoxin were barely detectable (0.06–0.09 ng/m³) because the wind direction during the 3-day beach study periods was offshore. During the exposure beach study of March 11–14, 2005, both the cell counts (59,000 to 200,000 cells/L) and the water concentrations of brevetoxins (2–12 µg/L) were at moderate levels, but the air concentrations of brevetoxin at Siesta Beach were moderate high at 20 to 38 ng/m³.

3.2. Inland transect studies

The analysis of total brevetoxin concentrations in the aerosol samples collected during the October 19 – 23, 2004 inland transect study indicated no detectable levels (<0.05 ng/m³) at any of the sample locations as shown in Table 2. The 5 day non-exposure data are not shown graphically since the levels of brevetoxin were below the limit of detection.

The total brevetoxin concentrations in the aerosol samples collected during the February 7 – 11, and March 15 – 19, 2005 inland transect studies were detectable, and are shown in Figures 2 and 3, respectively. Although the highest levels of total brevetoxins were always measured at the beach site (site A in Figure 1), brevetoxins were detected as far inland as 4.2 km from the beach (site E in Figure 1). The brevetoxin aerosol concentrations in general decreased with increasing distance from the Siesta Beach study site. As the aerosol was transported inland, it was dispersed in the air and some particles were deposited on the ground, resulting in decreased concentration from the beach (Y Chung, unpublished data).

4.0 Discussion

The results of this study demonstrate that brevetoxins were transported up to 6.4 km inland from the coastal water source. These data also illustrate the variability from day to day based on cell count (density and/or patchiness of the bloom), wind speed, and wind direction.

The significance of this transect study is that people may still be exposed environmentally to the aerosolized brevetoxins even after they leave the beach. Indeed, if people remain on a barrier island where no point is greater than 1.6 km from a coast, they will most likely be continuing their exposure in any outdoor setting and from all directions if the inland waters also contain *K brevis* blooms. This is significant as Fleming et al (2005, 2007, 2009) have demonstrated that asthmatics have significantly increased symptoms and decreased lung function after only a 1 hour exposure to aerosolized brevetoxins. Furthermore, aerosolized Florida red tide toxins seem to have more chronic effects, including symptoms and pulmonary function changes in asthmatics for several days following the 1 hour beach exposure (and/or environmental exposure inland), and increased admissions to emergency rooms for respiratory diseases (including pneumonia, bronchitis, and asthma) during active Florida red tides (Kirkpatrick et al., 2006, Kirkpatrick et al., 2009, Kirkpatrick et al., submitted). Currently, the public health message in communities with onshore *Karenia* blooms has only recommended leaving the beach area to avoid aerosol exposure; this message needs to be re-evaluated based on these new findings to take into account the possibility of inland environmental exposure to brevetoxins, particularly for persons with underlying lung diseases such as asthma.

4.1 Limitations

These findings are based on 1 non-exposure study and 2 exposure studies, therefore these data do not reflect the entire span of environmental conditions that may alter the distance the toxins travel. Environmental monitoring over a much longer time period is needed to fully understand the conditions that optimize inland transport, as well as monitoring even further inland. In addition, studies are needed to explore possible exposure to brevetoxins inside of buildings. Cell count data and water sampling and analysis for brevetoxins at the 2 sites located near the water would enhance the assessment of the bloom intensity and location.

5.0 Conclusions

This study has documented the transport of brevetoxins during Florida red tide blooms from the beach inland by placing a series of air samplers on an inland transect. This study is the first objective documentation of the transport of *K. brevis* brevetoxins inland. These findings should be considered by healthcare providers, public health officials and policy makers in communities affected by Florida red tide, particularly when making recommendations concerning the avoidance of brevetoxin exposure and health effects for persons with underlying lung disease.

Acknowledgments

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Abbreviations

PbTx	Brevetoxin
cm	Centimeter
<i>K. brevis</i>	<i>Karenia brevis</i>
km	Kilometer
LCMS	Liquid chromatography mass spectroscopy
ug/L	microgram per liter
ng/m ³	nanogram per meter cubed
NSP	neurotoxic shellfish poisoning

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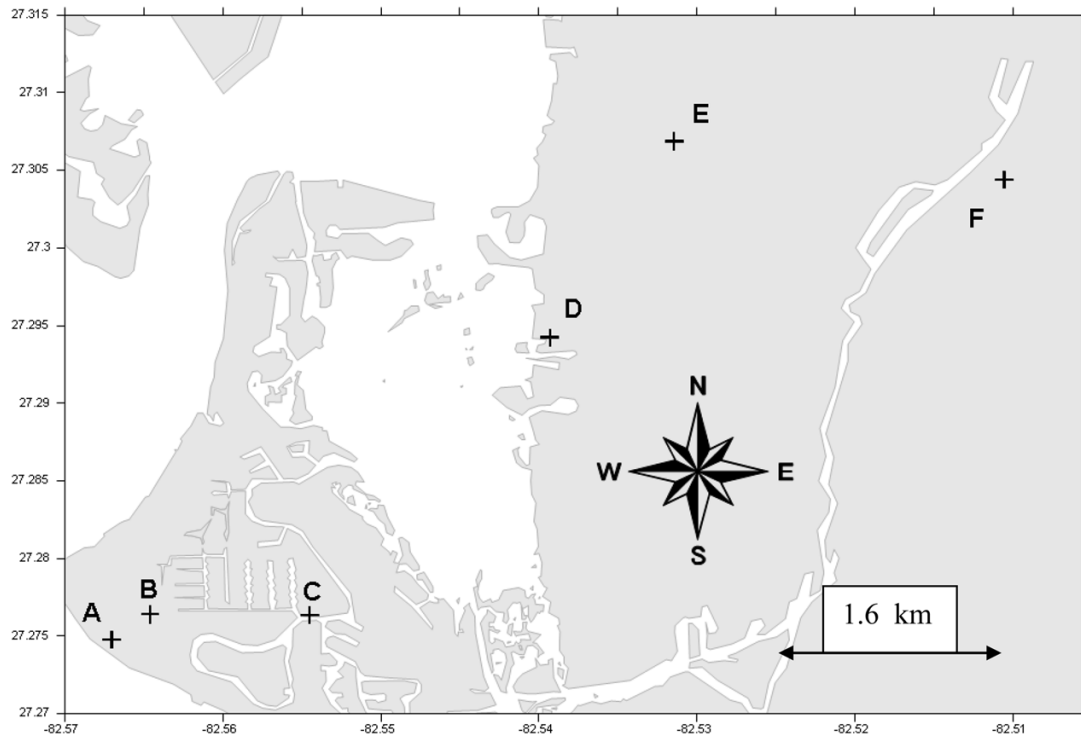


Figure 1.
Inland Transect Aerosol Sampling Locations

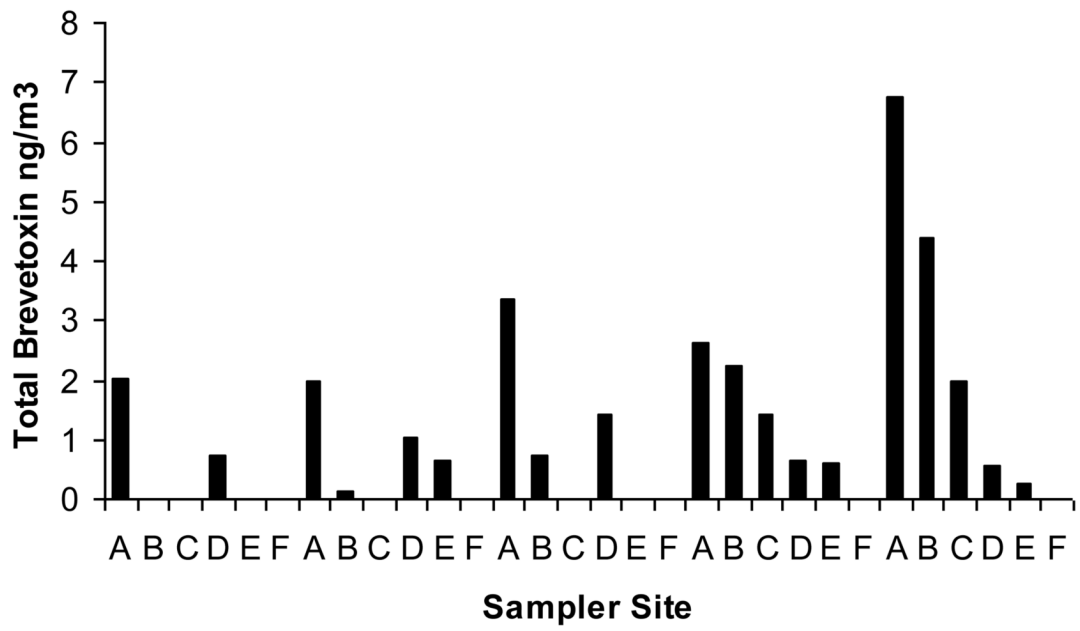


Figure 2.
Inland transect: February 2005

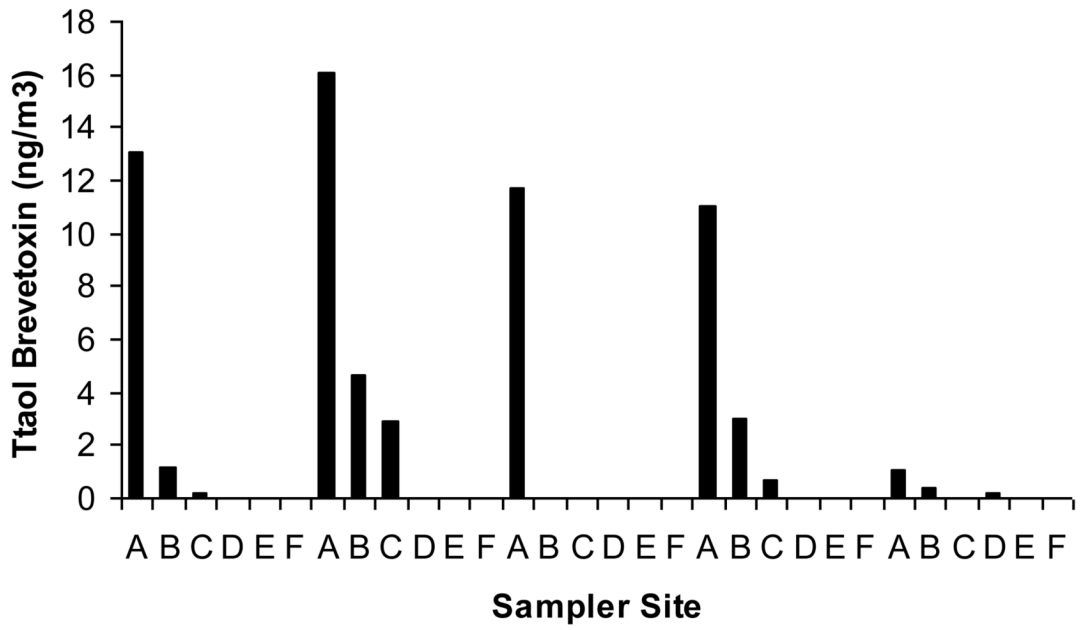


Figure 3.
Inland transect: March 2005

Table 1

Summary of Beach Studies: Environmental Data

Date	Temp. (° C)	Humidity (%)	Wind Speed (KPH)	Wind Direction	<i>K. brevis</i> in water (Cells/L) ¹	Water Toxin (ug/L)	Air Toxin (ng/m ³)
Non exposure beach study							
October 2004	16.0–27.7	35–91	0–18	Offshore to Onshore	< 1,000 to 2,000	< 0.03	< 0.05
Exposure beach Studies							
Feb 2005	8.9 – 23.9	40 – 97	0 – 24	Offshore	1,051,000 to 4,639,000	13 – 128	0.06 to 0.09
March 2005	16.7 – 21.0	14 – 98	0 – 19	Onshore	59,000 to 200,000	2 – 12	20 to 38

Table 2

Summary of 5 day Inland transect Studies: Environmental Data

Date	Temp. (° C)	Humidity (%)	Wind Speed (KPH)	Wind Direction	Air Toxin (ng/m ³)
Non exposure 5 day transect					
October 2004	19.4 – 28.3	50 – 91	0 – 26	Offshore to Onshore	< 0.05
Exposure 5 day transect					
Feb 2005	8.3 – 22.8	46 – 95	0 – 42	Offshore to Onshore	0.05 – 7.4
March 2005	10.0 – 22.2	43 – 95	0 – 32	Offshore to Onshore	0.05 – 16