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Florida Red Tide and Brevetoxins: Association and Exposure in Live Resident Bottlenose Dolphins (*Tursiops truncates*) in the Eastern Gulf of Mexico, U.S.A.

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Florida red tide and brevetoxins: Association and exposure in live resident bottlenose dolphins (*Tursiops truncatus*) in the eastern Gulf of Mexico, U.S.A.

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ABSTRACT

Bottlenose dolphins (*Tursiops truncatus*) along the Gulf of Mexico are frequently exposed to blooms of the toxic alga, *Karenia brevis*, and brevetoxins associated with these blooms have been implicated in several dolphin mortality events. Studies on brevetoxin accumulation in dolphins have typically focused on analyses of carcasses from large-scale die-offs; however, data are scarce for brevetoxin loads in live individuals frequently exposed to *K. brevis* blooms. This study investigated *in vivo* brevetoxin exposure in free-ranging bottlenose dolphins resident to Sarasota Bay, Florida, utilizing samples collected during health assessments performed during multiple *K. brevis* blooms occurring from 2003 to 2005. Brevetoxins were detected by ELISA and LC-MS in 63% of bottlenose dolphins sampled ($n = 30$) concurrently with a *K. brevis* bloom. Brevetoxins were present in urine and gastric samples at concentrations ranging from 2 to 9 ng PbTx-3 eq/g, and in feces at concentrations ranging from 45 to 231 ng PbTx-3 eq/g. Samples from individuals ($n = 12$) sampled during nonbloom conditions ($\leq$1,000 cells/L) were negative for brevetoxin activity. Brevetoxin accumulation data from this study complement dolphin carcass and prey fish data from the same study area, and aid in evaluating impacts of harmful algal blooms on sentinel marine animal species along the west Florida coast.

Key words: brevetoxin, *Karenia brevis*, bottlenose dolphin, *Tursiops truncatus*, harmful algal blooms, algal toxins, marine biotoxins, HAB, red tide.

One of the many natural stressors on coastal bottlenose dolphins (*Tursiops truncatus*) is exposure to harmful algal blooms (HABs). These blooms are concentrated assemblages of single-celled, photosynthetic marine algae that can naturally produce potent toxins and have been linked to several large-scale dolphin mortalities in U.S. coastal waters (Van Dolah *et al*. 2003). Dolphins can serve as sentinels of the health of coastal marine ecosystems and can bioaccumulate harmful substances due to their role as top predators (Wells *et al*. 2004, Bossart 2006), thus the impact of toxic phytoplankton blooms on dolphins has been a focus of investigation in recent years. One type of HAB that has had a large impact on dolphin communities along the U.S. coasts of the Atlantic Ocean and Gulf of Mexico is caused by the marine dinoflagellate *Karenia brevis*. HABs composed of *K. brevis* (commonly referred to as "Florida red tide" or simply "red tide") occur along Florida's Gulf of Mexico coast almost annually, can last for periods up to several months, and have negative impacts on wildlife and human health (Steidinger *et al.* 1998, Van Dolah 2000, Kirkpatrick *et al*. 2004).

K. brevis naturally produces a suite of potent neurotoxins called brevetoxins that interfere with normal neurological function by binding to voltage-gated sodium channels in neuronal cells (Ramsdell 2008). Brevetoxins produced by *K. brevis* consist of at least nine congeners, and several additional brevetoxin metabolites of varying toxicity have been identified from exposed organisms such as shellfish (Wang *et al.* 2004, Bottein-Dechraoui *et al.* 2007). Accumulation and trophic transfer of brevetoxins have been implicated in the deaths of a wide variety of wildlife species since the 1800s (for review, see Landsberg 2002) and recent studies show evidence of prey fish as brevetoxin vectors to bottlenose dolphins in the Gulf of Mexico (Flewelling *et al*. 2005; Naar *et al*. 2007; Fire *et al*., unpublished data).

Studies of the effects of red tides on dolphins have typically focused on brevetoxin accumulation in carcasses recovered during large-scale mortality events (Geraci 1989, Mase *et al*. 2000, Flewelling *et al*. 2005). One drawback of studying red tide exposure

in dolphins based solely on carcass data is the potential time lag between bloom exposure and the sampling of carcasses. Although the response to dolphin strandings is prompt and necropsies are often performed in a timely manner once carcasses are reported, in some cases days may pass between time of death and the time that carcasses wash up on a beach or are reported. The effects of decomposition on tissue concentrations of brevetoxin are not well known. While data from carcasses are useful for describing brevetoxin concentration ranges and tissue distribution, studies providing data on brevetoxin concentrations in live dolphins exposed to *K. brevis* blooms are lacking. Sampling live dolphins during a red tide would be a more appropriate approximation of real-time brevetoxin exposure levels. Of the various bottlenose dolphin research programs that have been involved in dolphin health assessments in U.S. waters in recent years, at least one has been operating in waters known to have frequent *K. brevis* blooms. Samples taken routinely from live dolphins during these health assessments include blood, feces, and urine (Wells *et al*. 2004). These sample types may be indicators of brevetoxin metabolism (Poli *et al.* 1990*a*, Benson *et al*. 1999) but are not always successfully recovered from necropsy sampling alone. Another advantage of sampling live dolphins to assess brevetoxin exposure is the availability of associated sighting histories for known individual dolphins, thus allowing determination on whether dolphins are "resident" to a given area. If a *K. brevis* bloom is present within a resident dolphin population's range, it can reasonably be assumed that those dolphins have had some exposure to brevetoxins.

This study investigates brevetoxin exposure of a resident dolphin population frequently exposed to *K. brevis* blooms, by focusing on brevetoxin accumulation in live members of the population. Investigating toxin exposure in live dolphins can further support toxin exposure data from dolphin prey fish and dolphin carcasses recovered in the same location (Fire *et al*. 2007, unpublished data). The primary objective of this study is to quantify brevetoxin levels in samples collected from live individuals of the Sarasota Bay area dolphin community during *K. brevis* blooms and also during periods of background *K. brevis* abundance. Here we present data from laboratory analyses of live dolphin samples taken during blooms of *K. brevis* as well as in periods when blooms were not present. Samples of plasma, serum, urine, feces, milk, and stomach contents were obtained from free-ranging bottlenose dolphins captured, sampled, and released in Sarasota Bay, Florida from June 2003 through February 2005. Collected samples were analyzed to determine levels of brevetoxins *via* enzyme-linked immunosorbent assay (ELISA) and liquid chromatography-mass spectrometry (LC-MS) methods.

METHODS

Sarasota Bay, Florida (27°N, 82°W), is a region of approximately 125 km^2, which is regularly inhabited by about 150 yr-round resident bottlenose dolphins (Scott *et al*. 1990, Wells 2003). Twice a year (February and June) between 2003 and 2005, the Sarasota Dolphin Research Program (SDRP) conducted health assessments on resident bottlenose dolphins inhabiting Sarasota Bay (Wells *et al*. 2004). This capture/release program was used as an opportunity to acquire samples from live bottlenose dolphins during *K. brevis* blooms, as well as during nonbloom conditions. Dolphins were captured by encircling them with a 500 m × 4 m seine net in shallow (<2 m) waters, using several small boats to insure the safe handling of the animals. Once secured, the dolphins were transferred onto the padded deck of a 9-m veterinary examination boat where a series of length and girth measurements were taken, as well as weight. Blood (~10 mL aliquot from a total of about 300 mL collected for a variety

of projects) was taken *via* venipuncture of the fluke by trained veterinary staff and was partitioned by centrifugation into plasma and serum for subsequent laboratory analysis. Urine samples were collected *via* the urethra using a sterile catheter. Milk was collected *via* a custom suction-tube collection system during dolphin processing. Gastric samples were collected using a small tube inserted *via* the esophagus into the stomach, from which stomach contents were drained and stored. Fecal samples were taken during processing using a sterile catheter. All samples were collected into sterile cryotubes or centrifuge tubes and stored at −80°C until analysis for brevetoxin activity using ELISA methods described below. With the exception of blood, sample volumes were variable for all sample types collected, depending on whether the dolphin had recently fed, voided, or was lactating.

Data on *K. brevis* cell counts in the study area were obtained from a database provided by the Mote Marine Laboratory Phytoplankton Ecology Program, which performs regular surveys (4–5 d/wk) of coastal waters near Sarasota, Florida. Additionally, phytoplankton samples were collected at the capture/release sites during dolphin processing, and viable *K. brevis* cells were enumerated using inverted light microscopy. Cell abundance data were analyzed with ArcView GIS 3.3 software (ESRI GIS and Mapping Software, Redlands, CA, USA) to visualize the abundance and distribution of *K. brevis* during blooms in the area, as well as during nonbloom periods. Water collection and dolphin sampling sites are shown in Figure 1.

For the purposes of defining the sample sets for this study, a "red tide event," or *K. brevis* bloom, was defined to be the sustained presence (spanning multiple sampling days at multiple stations) of intact *K. brevis* cells within the Sarasota Bay area and surrounding waters, at concentrations exceeding 5,000 *K. brevis* cells per liter of seawater (cells/L). As point of reference, 5,000 cells/L is used as the *K. brevis* abundance level triggering closure of shellfish harvesting areas in Florida waters (FDACS 2005). The "exposed" sample set of dolphins were resident individuals sampled within Sarasota Bay concurrent with a *K. brevis* bloom and consisted of 30 dolphins. The value for *K. brevis* abundance associated with each sampled dolphin was recorded as the maximum cell density in the area (within 30 km of the capture–release site) for the 7-d period prior to capture–release activity.

Four dolphins were sampled during a summer 2003 *K. brevis* bloom (9–10 June), 18 dolphins were sampled during a winter 2004 bloom (2–10 February), and 8 dolphins were sampled during a winter 2005 bloom (31 January–15 February). Samples recovered from dolphins during *K. brevis* blooms included plasma ($n = 27$), serum ($n = 27$), urine ($n = 24$), feces ($n = 9$), milk ($n = 3$), and stomach contents ($n = 7$). At least one sample type was recovered from each dolphin, yielding a sample set of 97 for the entire exposed dolphin group. An additional set of samples ($n = 12$ plasma, $n = 12$ serum, $n = 11$ urine, $n = 1$ milk) was taken from 12 dolphins captured and released in summer 2004 (2–11 June), a period with *K. brevis* abundance at or below baseline levels of 1,000 cells/L (Table 1). This sample set represents the "nonbloom" dolphins, sampled when there was no significant *K. brevis* presence, and after at least a 3-mo interval since the last local bloom. These samples were also analyzed for brevetoxin content by ELISA. A few dolphins were sampled during multiple capture/release sessions (FB118, FB188, FB220), but comparisons of brevetoxin concentrations among and between individuals were not pursued due to limited sample availability.

In the laboratory, brevetoxins were extracted from stomach contents and feces by homogenizing the sample in three volumes of acetone, followed by probe sonication for 2 min and centrifugation at 3,000 rpm for 3 min. The supernatant was evaporated

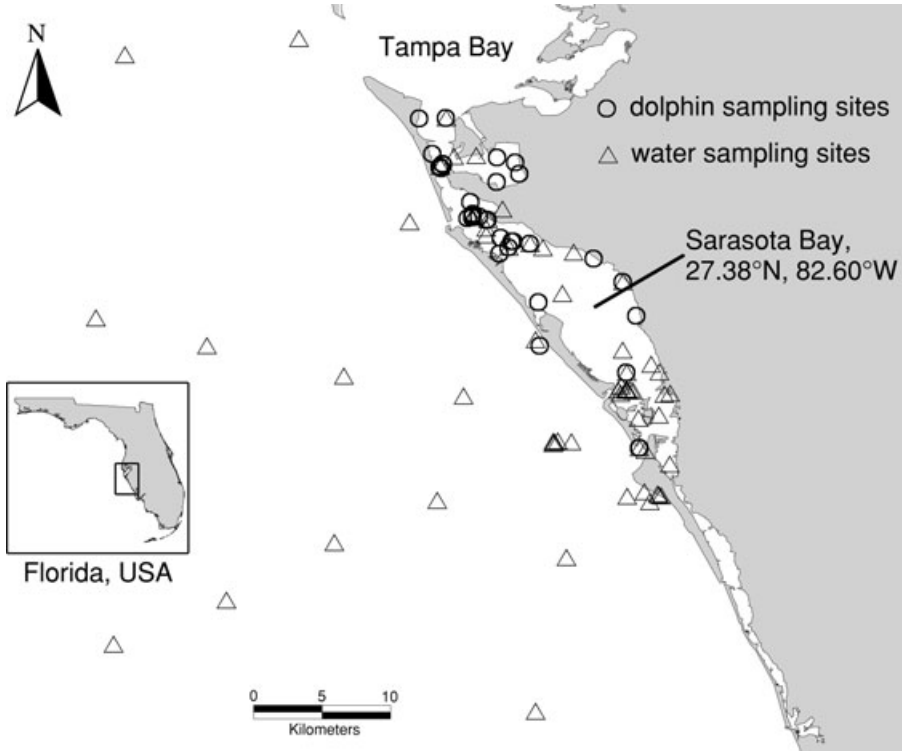

Figure 1. Sample collection sites in the Sarasota Bay area 2003–2005. Open triangles represent water-sampling sites used for *K. brevis* cell abundance; open circles represent capture/release sites for live dolphin samples.

and resuspended in 85% aqueous methanol, and solvent partitioned with hexane. The methanol fraction was collected and then passed through a 0.45-μm hydrophilic polypropylene syringe-driven filter (Pall Life Sciences, East Hills, NY, USA). The filtrate was again evaporated and resuspended in 100% methanol in preparation for analysis. Urine, plasma, and serum samples were centrifuged at 3,000 rpm and filtered (0.45 μm). Samples were further cleaned to reduce sample matrix effects by passing the extracts and aqueous samples through a preconditioned C-18 solid-phase extraction column under vacuum (Sigma-Aldrich Co., St. Louis, MO, USA). The C-18 columns were then rinsed with water and the sample was eluted with 100% methanol in preparation for analysis.

An ELISA was used as the primary method of brevetoxin quantification for all samples (Naar *et al*. 2002, Flewelling *et al*. 2005). This immunological assay utilizes antibrevetoxin antibodies to quantify the total brevetoxin-like molecules (both parent toxins and metabolites) in a sample, reported in brevetoxin-3 equivalents (PbTx-3 eq). Although this composite response does not distinguish among individual brevetoxin congeners, the ELISA is desirable due to its sensitivity at low concentrations and its utility as a reliable, high-throughput assay. All sample extracts were diluted prior to the assay to eliminate matrix effects (*i.e.*, nonspecific antibody binding and resultant false positives), which were confirmed to be negligible in uncontaminated

Table 1. Live dolphins sampled and *K. brevis* cell densities corresponding to time of dolphin sampling (maximum concentration for 7-d period prior to capture/release; cells/L of seawater).

Exposed group			Nonbloom group		
Dolphin ID	Date sampled	*K. brevis* cell density (cells/L)	Dolphin ID	Date sampled	*K. brevis* cell density (cells/L)
FB 118 (a)	6/9/2003	1.73×10^5	FB 181	6/2/2004	≤1,000
FB 79	6/9/2003	1.73×10^5	FB 230	6/2/2004	≤1,000
FB 128	6/10/2003	1.73×10^5	FB 185	6/3/2004	≤1,000
FB 198	6/10/2003	1.73×10^5	FB 92	6/3/2004	≤1,000
FB 178	2/2/2004	3.66×10^5	FB 138	6/4/2004	≤1,000
FB 188 (a)	2/2/2004	3.66×10^5	FB 196	6/4/2004	≤1,000
FB 224	2/2/2004	3.66×10^5	FB 148	6/7/2004	≤1,000
FB 27	2/2/2004	3.66×10^5	FB 114	6/8/2004	≤1,000
FB 7	2/2/2004	3.66×10^5	FB 20	6/8/2004	≤1,000
FB 173	2/3/2004	3.66×10^5	FB 188 (b)	6/10/2004	≤1,000
FB 177	2/3/2004	3.66×10^5	FB 99	6/10/2004	≤1,000
FB 33	2/3/2004	3.66×10^5	FB 159	6/11/2004	≤1,000
FB 9	2/3/2004	3.66×10^5			
FB 125	2/4/2004	3.66×10^5			
FB 175	2/5/2004	1.07×10^6			
FB 226	2/5/2004	1.07×10^6			
FB 2	2/9/2004	1.07×10^6			
FB 228	2/9/2004	1.07×10^6			
FB 65	2/9/2004	1.07×10^6			
FB 11	2/10/2004	1.07×10^6			
FB 118 (b)	2/10/2004	1.07×10^6			
FB 179	2/10/2004	1.07×10^6			
FB 135	1/31/2005	5.39×10^6			
FB 218	1/31/2005	5.39×10^6			
FB 232	2/1/2005	5.39×10^6			
FB 75	2/1/2005	5.39×10^6			
FB 187	2/2/2005	5.39×10^6			
FB 220 (a)	2/2/2005	5.39×10^6			
FB 189	2/4/2005	5.39×10^6			
FB 118 (c)	2/15/2005	9.94×10^5			
FB 220 (b)	2/15/2005	9.94×10^5			

samples. The ELISA method used in this study has a limit of quantification of 5 ng PbTx-3 eq/g for stomach contents and feces, and 1.2 ng PbTx-3 eq/mL for urine, plasma, milk, and serum.

Selected ELISA-positive samples ($n = 15$ urine, $n = 2$ gastric, $n = 2$ feces) underwent confirmatory analysis by LC-MS methods. LC-MS discriminates between individual molecules by their ionic mass-to-charge ratios and can unambiguously determine the presence of known brevetoxins (for which standards are available) in a given sample. Selected samples were initially analyzed for PbTx-3 following LC-MS methods described by Plakas *et al*. (2004), using a ThermoFinnigan AqA

HPLC-MS equipped with an AqA single quadrupole system. The LC consisted of a SpectraSystems LC Pump P4000, Autosampler AS3000, and a Degasser SCM1000. Mass spectral detection was obtained using an AqA single quadrupole system scanned from 204 to 1216 AMU with AqA Max 40 V, and a scan rate of 1.1 scans/s, with a limit of quantification of 300 ng/mL. Further analysis of additional brevetoxin congeners, including known metabolites, was performed using an Agilent 1100 LC system coupled to an Applied Biosystems/MDS Sciex 4000 Q TRAP hybrid triple quadrupole/linear ion trap mass spectrometer equipped with a Turbo V ionization interface. All LC separation was performed on a Phenomenex Luna C8(2), 5 μ, 2.0 × 150 mm column with a water (A)/acetonitrile (B) containing 0.1% acetic acid additive gradient. Analytes were detected in positive ion mode using the multiple reaction monitoring (MRM) method, covering the following brevetoxin congeners: PbTx-1, -2, -3, -7, -9, hydrolysis products of PbTx-3 and 7, cysteine-PbTx-B (A) and its sulfoxide (Wang *et al.* 2004, Abraham *et al.* 2006). The signal-to-noise ratio was ~29 for injection of 5 μL of 1 ng/mL of PbTx-3 for LC-MS analysis.

RESULTS

Weekly maximum *K. brevis* cell concentrations in the study area during capture, sampling, and release of the exposed dolphin group ranged from 1.7×10^5 cells/L to 5.4×10^6 cells/L (Fig. 2). *K. brevis* cell concentrations taken immediately next to the processing boats at the time of dolphin capture–release reached as high as

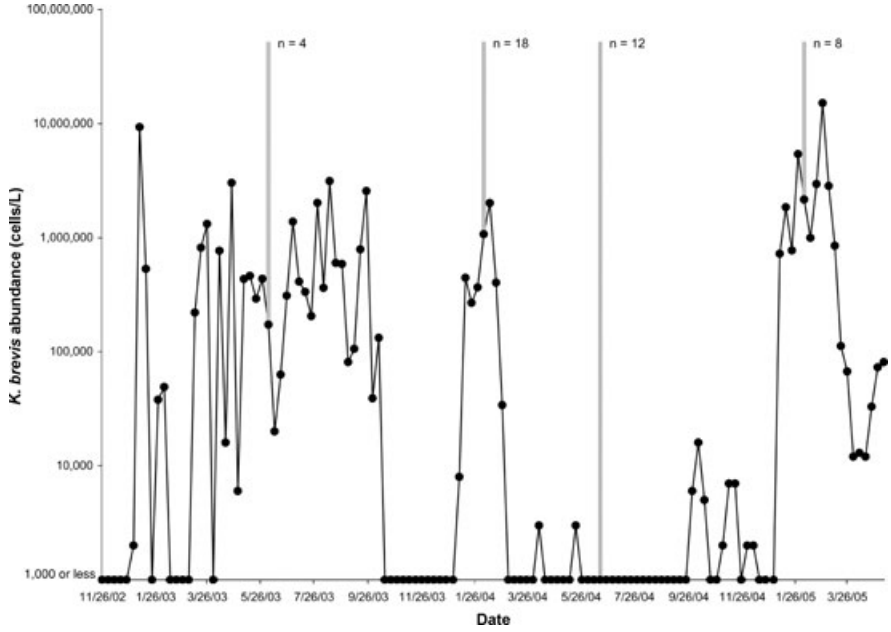

Figure 2. Weekly maximum *K. brevis* cell concentrations for Sarasota Bay and adjacent waters, 2003–2005. Grey bars indicate live dolphin sample collections during *K. brevis* blooms (summer 2003, winter 2004, winter 2005) and during a nonbloom period (summer 2004).

4×10^5 cells/L. In fact, during some dolphin capture–release sessions, many of the research staff and volunteers experienced mild-to-moderate respiratory irritation while standing in the water and handling the dolphins, consistent with the associated aerosolized brevetoxin component common to red tide events (Pierce 1986). All *K. brevis* cell concentrations occurring during capture, sampling, and release of nonbloom dolphins were at background levels ($\leq$1,000 cells/L).

ELISA analysis of exposed dolphin samples yielded detectable levels of brevetoxin activity in 19 of the 30 individuals tested. Of the 97 samples of varying matrices taken from these dolphins, 34 had a positive ELISA brevetoxin response, with concentrations ranging between 2 and 231 ng PbTx-3 eq/g (Table 2). The highest

Table 2. Distribution of ELISA brevetoxin activity (ng PbTx-eq/g or mL) detected in live dolphins sampled during *K. brevis* blooms in Sarasota Bay, 2003–2005.

	Sample type					
Dolphin ID	Urine	Plasma	Serum	Feces	Gastric	Milk
FB 118 (a)	<dl					
FB 79	2*					
FB 128	<dl					
FB 198	<dl					
FB 178		<dl	<dl	50		
FB 188 (a)	<dl	<dl	<dl	231		
FB 224		<dl	<dl			
FB 27	3*	<dl	<dl			
FB 7	4*	<dl	<dl	51		
FB 173		<dl	<dl			
FB 177		<dl	<dl			
FB 33	2	<dl	<dl			<dl
FB 9	<dl	<dl	<dl	214		<dl
FB 125	2*	<dl	<dl			
FB 175	2*	<dl	<dl	161		
FB 226	5*	<dl	<dl			
FB 2	4*	<dl	<dl			
FB 228	<dl	<dl	<dl			
FB 65	2	<dl	<dl			
FB 11	2	<dl	<dl	148		
FB 118 (b)	4	<dl	<dl	160		
FB 179	2*	<dl	<dl	45		
FB 135	<dl	<dl	<dl		<dl	
FB 218	<dl	<dl	<dl		<dl	
FB 232		<dl	<dl			
FB 75	4*	<dl	<dl		<dl	<dl
FB 187		<dl	<dl			
FB 220 (a)	<dl	<dl	<dl	<dl	9	
FB 189		<dl	<dl		<dl	
FB 118 (c)	2*	<dl	<dl		9*	
FB 220 (b)	4	<dl	<dl		<dl	
PbTx-positive samples	15/24	0/27	0/27	8/9	2/7	0/3
PbTx-positive dolphins	19/30					

<dl = below detection limit. Asterisks (*) denote samples confirmed positive by LC-MS.

brevetoxin activity was found in fecal samples, and eight of nine fecal samples analyzed had a positive ELISA brevetoxin response. The majority of urine samples analyzed were also positive for brevetoxin activity (15 of 24 samples). Gastric samples taken from the dolphins tested positive for brevetoxin activity in two cases (out of seven collected). Brevetoxin activity was not detected in plasma, serum, or milk samples from the exposed group, and was not detected in any samples collected from nonbloom dolphins.

LC-MS analyses of ELISA-positive samples from exposed dolphins unambiguously confirmed the presence of parent brevetoxins (PbTx-3) and/or known brevetoxin metabolites (hydrolysis product of PbTx-7, hydrolysis product of PbTx-3) in 11 of 19 samples tested (Table 3). The total quantifiable brevetoxin content for these samples (parent PbTx and quantifiable metabolites) ranged in concentration from 0.1 to 2.2 ng PbTx/mL sample.

All of the dolphins sampled were examined by experienced marine mammal veterinarians, and were found to exhibit a relatively typical suite of conditions. No life-threatening conditions were observed for any of the exposed dolphins, and all were observed alive well after the time of sampling. Some of the exposed individuals presented with a variety of active or healing lesions, but the cases were minor. Thirty of the exposed dolphins were sampled for morbillivirus, and all were negative. Leucocyte counts were within normal range (5,600–12,400 cells/μL; Bossart *et al*. 2001) for 22 of 30 exposed dolphins sampled, and ranged from 12,700 to 16,800 cells/μL for the eight other individuals.

Table 3. Brevetoxin congeners detected by LC-MS (ng/g or ng/mL) in ELISA-positive samples from live dolphins sampled during *K. brevis* blooms in Sarasota Bay, 2003–2005.

Dolphin ID	Sample type	PbTx-3	Hydrolysis product of PbTx-3	Hydrolysis product of PbTx-7
FB 79	Urine	0.1	0.4	<dl
FB 7	Urine	0.1	<dl	pos.
FB 27	Urine	0.6	<dl	<dl
FB 178	Feces	<dl	<dl	<dl
FB 33	Urine	<dl	<dl	<dl
FB 125	Urine	<dl	<dl	pos.
FB 226	Urine	0.8	1.4	<dl
FB 175	Urine	<dl	0.2	pos.
FB 2	Urine	0.2	0.2	pos.
FB 65	Urine	<dl	<dl	<dl
FB 179	Feces	<dl	<dl	<dl
FB 11	Urine	<dl	<dl	<dl
FB 118 (b)	Urine	<dl	<dl	<dl
FB 179	Urine	<dl	0.2	<dl
FB 75	Urine	0.1	<dl	pos.
FB 220 (a)	Gastric	<dl	<dl	<dl
FB 118 (c)	Urine	0.2	0.5	pos.
FB 118 (c)	Gastric	0.4	<dl	<dl
FB 220 (b)	Urine	<dl	<dl	<dl

<dl = below detection limit. pos. = qualitatively determined to be present.

Discussion

The *K. brevis* blooms associated with sampling of dolphins (exposed group) during summer 2003, winter 2004, and winter 2005 had cell densities exceeding 1×10^5, 1×10^6, and 5×10^6 cells/L, respectively (Table 1). Such cell densities are indicative of moderate-to-severe red tide blooms, and thus indicative of a significant potential exposure to brevetoxins. The dolphins' temporal and spatial proximity to elevated *K. brevis* cell densities during sampling, combined with detection of brevetoxin by ELISA and LC-MS in various samples from the exposed group, provides strong evidence that live dolphins of the Sarasota Bay area resident population accumulate brevetoxins when exposed to *K. brevis* blooms.

Brevetoxins were detected in gastric samples from 29% of exposed dolphins, suggesting that toxin uptake *via* trophic transfer does occur in this dolphin community. The bottlenose dolphins of Sarasota Bay area are strictly piscivorous (Barros and Wells 1998), and during *K. brevis* blooms brevetoxins are present in their primary prey items, reaching concentrations of 7,472 and 10,0844 ng PbTx-eq/g in fish tissues and stomach contents, respectively (unpublished data). Other routes of brevetoxin exposure are possible, however, such as incidental ingestion of dissolved toxin in seawater, or inhalation of airborne brevetoxin particles (Bossart *et al.* 1998, Pierce *et al*. 2005), but the high concentrations reported in prey fish and in stomach contents of dolphin carcasses strongly indicate trophic transfer as the likely primary exposure route. The absence of brevetoxin in plasma and serum samples is consistent with dosing studies showing much lower concentrations detected in blood compared with other sample types such as urine (Radwan *et al*. 2005).

Brevetoxin was detected in over 50% of the exposed group urine samples and in nearly all exposed group fecal samples. This is an indication that at the time of sampling, the dolphins were depurating and eliminating some quantity of toxin regardless of its exposure pathway, consistent with data from brevetoxin dosing studies in rats, which indicate a high degree of fecal and urinary excretion of the toxin within the first 48 h of exposure (Poli *et al.* 1990*b*, Cattet and Geraci 1993, Radwan *et al*. 2005). In these laboratory dosing studies, feces was the major route of clearance, along with a minor urinary component. However, the individuals sampled in this study were likely to have been exposed over longer periods of time, in contrast to the single-dose exposure used in these laboratory experiments, and may have been eliminating toxin continually as it was introduced to the dolphin in successive feeding events.

The brevetoxin concentrations detected in this study were very low in comparison to those associated with large-scale dolphin mortality events, where maximum values in urine, feces, and stomach contents reached 237, 774, and 12,151 ng/g, respectively (Flewelling *et al.* 2005; M. Twiner, unpublished data). Although the actual brevetoxin dose received by dolphins in this study is unknown, all individuals were asymptomatic for brevetoxicosis during capture and appeared in good health. The detection of brevetoxins at these low levels was also a factor in LC-MS confirmation of brevetoxin in only 58% of ELISA-positive samples. Although ELISA and LC-MS brevetoxin detection methods are robust and correlate well in other field studies (Pierce *et al.* 2006, Naar *et al.* 2007), ELISA brevetoxin values in this study approached the LCMS detection limit, resulting in a decreased probability of detection of individual brevetoxin congeners in the samples.

Brevetoxins were not detected in any of the nonbloom dolphin samples. This is consistent with the lack of a detectable bloom of *K. brevis* in the study area at the

time the nonbloom dolphin group was sampled. Brevetoxins have been reported to be present in Sarasota dolphin prey items during nonbloom conditions (unpublished data); however, these data indicate that associated brevetoxin levels in live dolphins are not detectable in spite of potential food web exposure.

The high abundance of *K. brevis* in water samples collected concurrently with detectable brevetoxins (parent toxins and metabolites) in dolphin urine and feces indicates recent exposure to, and excretion of brevetoxins. However, this exposure was not associated with a spike in dolphin mortalities similar to those seen in large dolphin die-offs such as that occurring in St. Joseph Bay, Florida, in 2004 (Flewelling *et al*. 2005), and all brevetoxin-positive dolphins in this study were still alive at the conclusion of this study. In fact, the question has been raised as to whether dolphin populations not commonly exposed to red tide (*i.e.*, St. Joseph Bay) are more susceptible to high mortality than populations that frequently experience them (*i.e.*, Sarasota Bay) (Van Dolah 2005). Follow-up studies of brevetoxin exposure in Florida Gulf coast dolphins may do well to investigate differences between dolphin populations, including factors such as the ratio of transient *vs.* resident dolphins, or genetic or predisposing health factors that may potentially increase susceptibility to red tide toxins.

Conclusions

The results from this study provide the first live animal *in vivo* exposure data for bottlenose dolphins. Dolphins present in *K. brevis* blooms have detectable levels of brevetoxin-like compounds, and the positive ELISA response in fecal and urine samples indicates that these compounds are being depurated from their bodies throughout the bloom. While previous studies have shown brevetoxins to be detectable in stomach contents and tissues from dolphin carcasses recovered during or after *K. brevis* blooms (Mase *et al*. 2000, Flewelling *et al*. 2005, Fire *et al*. 2007), data from this study show real-time exposure of dolphins affected by red tide. Data from this study complement dolphin carcass and prey fish brevetoxin data from Sarasota Bay dolphins (Fire *et al*. 2007, unpublished data) adding another layer of evidence in establishing a relationship between *K. brevis* blooms and their impacts on bottlenose dolphins. Further, the lack of brevetoxin detected during nonbloom conditions indicates that background toxin exposure in live dolphins is absent or below our current limit of quantitation (because prey fish can vector detectable levels of brevetoxins several months postbloom; Naar *et al*. 2007; Fire *et al*., unpublished data), and this study provides the first data on baseline toxin values for live specimens of the Sarasota Bay dolphin community.

Further studies involving the analysis of brevetoxin in live samples of bottlenose dolphins may also benefit from an improved understanding of gut and kidney clearance times for these animals (Malvin *et al*. 1971, Ridgway 1972, Malvin *et al*. 1978). In other piscivorous marine mammals, fish are completely digested within a 12-h period following feeding (Bigg and Fawcett 1985). The ability to determine how quickly a brevetoxin-containing food item is digested and how quickly the toxins are released into the bloodstream from the digestive tract may be important in relating toxin values in gastric and blood toxin samples. Additional studies analyzing the distribution of brevetoxin in live dolphins will likely need to utilize a combination of analytical techniques to give a broader understanding of the effects of this toxin on the health of wild bottlenose dolphins.

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