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Brevetoxin-associated mass mortality event of bottlenose dolphins and manatees along the east coast of Florida, USA

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ABSTRACT: A mass mortality of bottlenose dolphins *Tursiops truncatus* and Florida manatees *Trichechus manatus latirostris* co-occurred with a severe bloom of the toxic algal species *Karenia brevis* along the eastern coast of Florida, USA, between October 2007 and January 2008. Brevetoxin (PbTx), a potent neurotoxin produced by this marine alga, was detected in 69 and 92% of the tested carcasses of manatees and dolphins, respectively, at concentrations similar to those reported for earlier mortality events along the west coast of Florida. Brevetoxin was also detected in fetal and neonate dolphins, providing evidence of maternal transfer of the toxin in wild populations. This study is the first to document a brevetoxin-associated marine mammal mortality event along the Atlantic coast of Florida. It also demonstrates that, despite the rarity of *K. brevis* blooms in this region, significant negative impacts to marine mammals inhabiting this region can occur.

KEY WORDS: Brevetoxin · Harmful algal bloom · Dolphin · Manatee · Mortality HAB · Indian River Lagoon

INTRODUCTION

Free-ranging marine mammals in coastal waters of the United States face significant threats to individual health and population status. Some marine mammal species are apex predators and so also serve as sentinel species, so that knowledge of observed changes to marine mammal health can also indicate the state of our oceans' health (Bonde et al. 2004, Wells et al. 2004, Bossart 2006). In addition to common anthropogenic health stressors such as contaminants, ship strike and fishery interaction (O'Shea et al. 1984, Laist et al. 2001, Read et al. 2006, Balmer et al. 2011), natural processes such as harmful algal blooms (HABs) also pose a serious threat to marine mammal conservation, and exposure to severe HABs frequently leads to mass mortality events (Landsberg et al. 2005, Fire & Van Dolah 2012). Prominent among HABs that cause marine mammal die-offs are blooms of the toxic dinoflagellate *Karenia brevis*, which occur almost annually in Florida coastal waters and can last for several months (Steidinger et al. 1998, Heil & Steidinger 2009). *K. brevis* produces a suite of potent neurotoxins known as brevetoxins, or PbTxs (Steidinger et al. 1998). These toxins accumulate in the planktonic and benthic food webs, concentrating in prey organisms such as finfish or on seagrasses, which then act as vectors of the toxins to dolphins and manatees that feed upon them, resulting in dietary exposure and brevetoxicosis (Fle-

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welling et al. 2005, Fire et al. 2008). Bottlenose dolphins *Tursiops truncatus* (hereafter referred to as 'dolphins') and Florida manatees *Trichechus manatus latirostris* (hereafter referred to as 'manatees') inhabiting Florida's Gulf coast have been particularly impacted by *K. brevis* blooms, with frequent mass mortalities associated with brevetoxin exposure since the 1940s (Gunter et al. 1948, Bossart et al. 1998, Landsberg & Steidinger 1998, Mase et al. 2000, NMFS 2004, Fauquier et al. 2005, Flewelling et al. 2005, Landsberg et al. 2009, Twiner et al. 2012).

Although *K. brevis* is endemic to nearly all Florida coastal waters, brevetoxin-producing blooms are rare along the Atlantic coast and associated mass mortality of bottlenose dolphins or manatees has not been reported in this region (Tester & Steidinger 1997, Heil & Steidinger 2009). There are several populations of bottlenose dolphins that inhabit the Atlantic coast of Florida, with a resident Indian River Lagoon (IRL) estuarine system stock as well as 3 oceanic stocks (NOAA 2013). Manatees also inhabit much of the Atlantic coast, and are also found within the IRL (USFWS 2009). The present study describes the first reported mass mortality event of bottlenose dolphin or manatees along the east coast of Florida associated with brevetoxin exposure. Here we present data collected during this 2007 multi-species die-off occurring along Florida's Atlantic coastal and inshore waters and its co-occurrence with a severe toxic bloom of *K. brevis*. We present evidence of brevetoxin exposure as the presumed cause of death in both dolphins and manatees and discuss the relevance of this event to similar mass mortality events occurring previously in Florida coastal waters.

MATERIALS AND METHODS

Dolphin sample collection

All dolphins recovered during this mortality event had stranded or were sampled between 12 and 27 December 2007. All dolphins sampled in the present study (n = 14; 12 carcasses, 2 live animals) were recovered from Atlantic oceanside beaches between New Smyrna Beach and Palm Bay, Florida, USA (Volusia and Brevard counties, 29.013008° to 28.025153° N) except for Hubbs-0783-Tt, which was recovered in the Banana River area, and Hubbs-0778-Tt and Hubbs-0779-Tt, which were live animals entrapped in a small shallow area of the Mosquito Lagoon encircled by spoil islands and sandbars. These 2 dolphins, which were exhibiting no signs of brevetoxin exposure or other health effects, were relocated by boat to deeper water in the lagoon. Standard blood collection (Geraci & Lounsbury 2005) was made prior to release for hematologic evaluation and brevetoxin analysis due to the dolphins' proximity to the die-off area. Dolphin carcasses were either necropsied on site or transported to the laboratory (Hubbs-SeaWorld Research Institute, Orlando, Florida, USA) for comprehensive necropsy according to established protocols (Geraci & Lounsbury 2005). During necropsy and live sampling, gross pathological findings and morphometric data were recorded, and biological samples were collected for analysis. Dolphins were categorized by sex and age classes using standardized measurements of total length (Geraci & Lounsbury 2005) and age class grouping methods outlined in Wells et al. (1987); ≥246 cm for adult males, ≥231 cm for adult females, 161–245 cm for juvenile males, 161–230 cm for juvenile females, and ≤160 cm for calves. Dolphin tissues (300–500 g per sample) and fluids (2–10 ml per sample) were collected for brevetoxin testing and included samples of stomach contents, feces, liver, lung, blood, muscle, spleen, kidney, blubber, eye and urine. Samples for brevetoxin analysis were collected into polypropylene sample tubes or plastic bags and were stored at −20°C until toxin extraction.

Manatee sample collection

All manatees recovered during this mortality event were reported between 4 October 2007 and 12 January 2008. Manatee carcasses were either necropsied on site or transported by trailer to the Florida Fish and Wildlife Conservation Commission's (FWC) Marine Mammal Pathobiology Laboratory (MMPL) in Saint Petersburg, Florida, USA, for comprehensive necropsy and sample collection. At the MMPL, carcasses were refrigerated overnight until necropsy the following morning. All manatee carcasses in the present study (n = 33) were recovered between Ormond Beach and Fort Pierce (Volusia, Brevard, Indian River and St. Lucie counties, 29.293400° to 27.472102° N) within the Indian River Lagoon (IRL; inclusive of Indian River, Banana River and Mosquito Lagoon). During necropsy, gross pathological findings, gross observations of gastrointestinal contents and morphometric data were recorded, and samples of stomach contents, ingested tunicates, feces, liver, lung, kidney, urine, milk and muscle were collected (n = 30 individuals) for analysis. Manatees were categorized by age class based on methods outlined in

O'Shea & Langtimm (1995); >265 cm for adults, 236–265 cm for sub-adults, and <236 cm for calves. Samples for brevetoxin analysis were collected into polypropylene sample tubes or plastic bags and were stored at −20°C until toxin extraction.

Phytoplankton collection and analysis

Phytoplankton samples were collected as part of separate ongoing statewide monitoring and event response efforts routinely conducted by FWC. These phytoplankton data (n = 718 samples; 6 September 2007 to 22 January 2008) were queried from the FWC Harmful Algal Bloom Monitoring Database to obtain concentrations of *Karenia brevis* in seawater samples, reported as cells of *K. brevis* per liter of seawater (cells l^{-1}).

Toxin extraction

Urine and serum samples were centrifuged at 3000 × *g* for 10 min and the supernatants collected prior to analysis. All other samples were homogenized in 80% methanol (4 ml g^{-1}), heated in a hot water bath at 60°C for 20 min, and centrifuged at 3000 × *g* for 10 min. The supernatant was retained, and the pellet was extracted a second time in the same manner. The supernatants were pooled per tissue sample and brought to a final volume of 10 ml with 80% methanol. The extract was partitioned once with 100% hexane (1:1, v:v), and the methanol fraction was retained. In 2 manatees, samples of seagrass and associated tunicates were opportunistically sub-sampled from stomach contents and extracted using the above methods. Extracts for all samples were stored in glass vials at −20°C until analysis.

ELISA analysis

Extracts were analyzed primarily by enzyme-linked immunosorbent assay (ELISA) methods, according to procedures described in Naar et al. (2002). The ELISA uses cross-reactivity of brevetoxin to anti-brevetoxin antibodies to determine brevetoxin-like activity in a sample. Quantitation is determined via competition between brevetoxin in the sample and brevetoxin bound to a 96-well plate for binding to anti-brevetoxin antibodies. Antibodies that bind to the plate are then visualized by recognition by a secondary antibody linked to an enzyme that catalyzes a colorimetric reaction. Concentrations are reported as PbTx-3 equivalents and reflect the overall concentration of brevetoxin and brevetoxin-like compounds present in the sample. Recovery of PbTx-3 standard reference material (AgResearch, Hamilton, New Zealand) added to negative control samples (brevetoxin-negative dolphin and manatee tissues; liver, muscle, brain, lung) was between 94 and 108% using this method. The detection limits for this assay in the present study were approximately 1 ng ml^{-1} for urine and serum samples and 5 ng g^{-1} for all other samples.

LC-MS analysis

Selected assay-positive extracts were cleaned by C18 solid-phase extraction cartridges (Bond Elut, 500 mg; Agilent) and analyzed by liquid chromatography-mass spectrometry (LC-MS) for parent PbTx toxins and metabolites. Sample preparation and analyses were carried out according to methods described in Fire et al. (2011). Liquid chromatographic separations were performed on a Luna C8(2) 150 × 2 mm column using an Agilent Technologies Model 1100 LC system. Mobile phase consisted of water (A) and acetonitrile (B), with 0.1% formic acid additive with a gradient elution. The mobile phase flow rate was 0.2 ml min^{-1}. The eluate from the LC was analyzed by an AB Sciex 4000 QTRAP hybrid triple quadrupole/linear ion trap mass spectrometer equipped with a TurboV™ interface (Foster City, CA, USA). Analysis of brevetoxin congeners and metabolites by mass spectrometry was achieved by multiple reaction monitoring. Samples were screened for PbTx-3 (dihydrobrevetoxin-B), PbTx-7 (dihydrobrevetoxin-A) and PbTx-9 (tetrahydrobrevetoxin-B), hydrolysis products of PbTx-3 and PbTx-7, and cysteine adducts of PbTx-A (brevetoxin-A) and PbTx-B (brevetoxin-B) and their sulfoxides. The detection limits were 0.2, 2.6, 1.0, and 4.3 ng per gram sample for PbTx-3, PbTx-7, S-desoxybrevetoxin-B2, and brevetoxin-B2, respectively, for toxin standards prepared in methanol.

RESULTS

Association of strandings with *K. brevis* bloom

The 2007 Florida east coast bloom of *Karenia brevis* was first detected in the northern portion of the study area (Nassau to Flagler counties, Fig. 1), with high cell concentrations exceeding 1 000 000 cells l^{-1} first re-

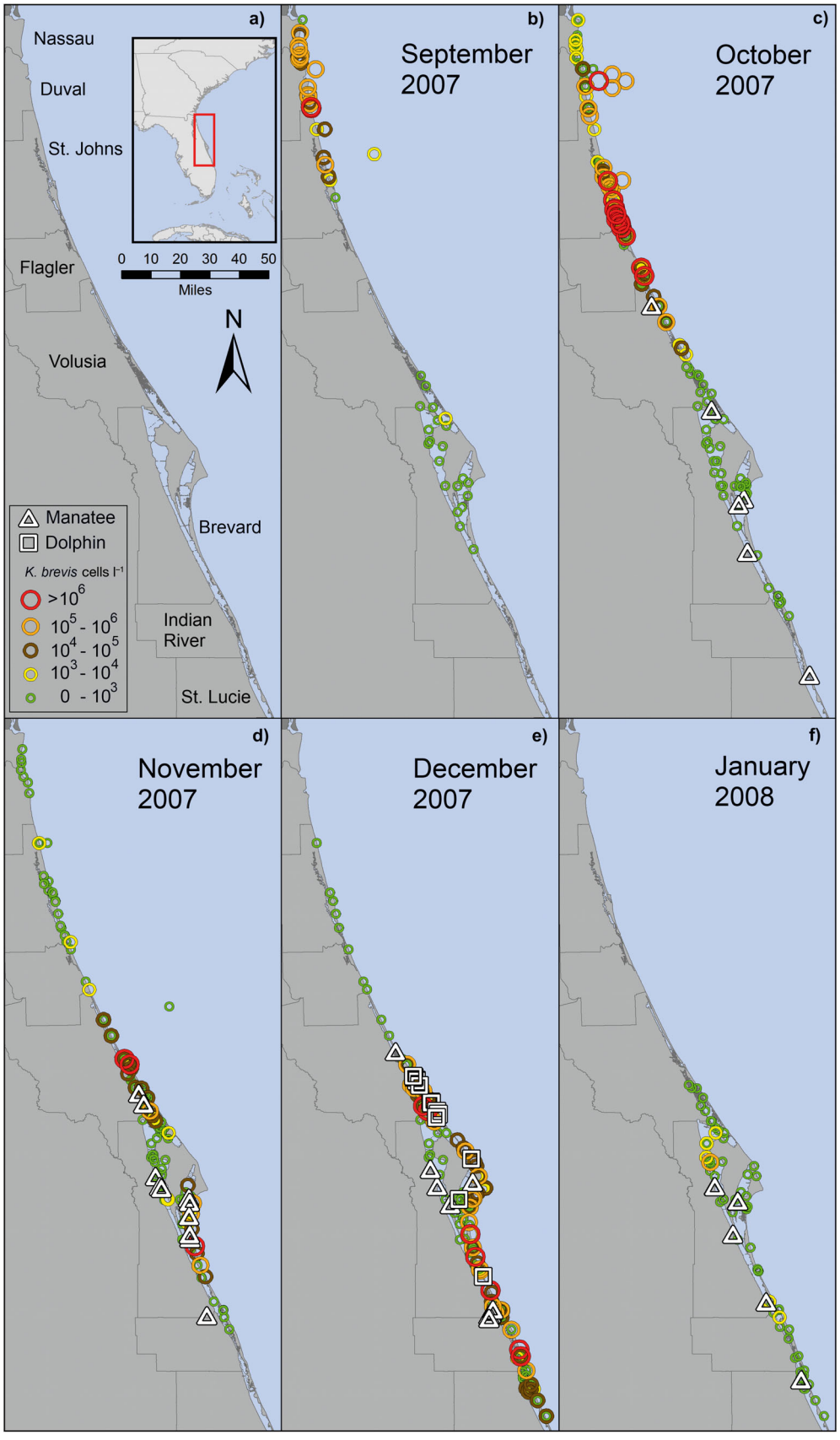

Fig. 1. (a) Study area of Nassau County to St. Lucie County, Florida, USA, with locations of bottlenose dolphin *Tursiops truncatus* and manatee *Trichechus manatus latirostris* strandings and phytoplankton sampling efforts during (b) September 2007, (c) October 2007, (d) November 2007, (e) December 2007, and (f) January 2008

ported 26 September (Fig. 2). By 16 October, *K. brevis* cell concentrations had reached a maximum of 6 030 000 cells l^{-1}. The appearance of high cell counts progressed southward along the coast, while decreasing steadily in concentration (eventually reaching ~10 000 cells l^{-1}) until mid-November. On 20 November *K. brevis* cell concentrations again dramatically increased to more than 1 000 000 cells l^{-1}, with cell counts reaching a peak of 5 693 000 cells l^{-1} on 1 December. By this time the resurgent bloom had spread farther southward, and by 9 December had reached the region where all of the manatee and dolphin strandings occurred (Fig. 1). Cell densities in excess of 100 000 cells l^{-1} were observed throughout this region until 9 January 2008, then declined to background concentrations (≤1000 cells l^{-1}) by 15 January 2008, having spread over a linear distance of ~250 miles (ca. 400 km) since the bloom began in September. Overall, high cell concentrations throughout the bloom were detected at both inshore and beachside sampling sites, as well as in the passes.

Beginning on 4 October 2007, single manatee carcasses were reported in the IRL, and reports of dead manatees continued until 12 January 2008. Temporally, the strandings were uniformly distributed throughout this period (Fig. 2). Geographically, most strandings were clustered at the confluence of the Mosquito Lagoon, Indian River and Banana River immediately inshore of Cape Canaveral, covering a linear distance of ~135 miles (ca. 220 km) across Volusia, Brevard, Indian River and St. Lucie counties (Fig. 1). In October, 5 manatee strandings occurred well before the bloom had reached the region where the strandings were reported. Additional manatee strandings (n = 5) adjacent to our study region occurred during the September 2007 to January 2008 period, but these were animals that were recovered inland along the St. Johns River or its tributaries, and so were not included as part of our sample set. The dolphin strandings were much more strongly temporally clustered than the manatees, with 10 of the 12 carcasses recovered within a 4 d period (12 to 15 December). Spatially, the extent of strandings was also smaller, covering a linear distance of ~75 miles (ca. 120 km) across Volusia and Brevard counties and primarily grouped along the Atlantic beaches adjacent to the Mosquito Lagoon. All dolphins, including the live dolphins, were sampled within the time frame of the second bloom peak (approx. 20 November 2007 to 9 January 2008).

Dolphin analyses

Based on total body length recorded at necropsy or capture, the age classes of the dolphins sampled were distributed as follows: 5 adults (3 males, 1 female, 1 unknown), 7 juveniles (5 males, 1 female, 1 unknown), 1 male neonate calf and 1 male fetus (Table 1). Sex class was skewed toward males, with 10 male dolphins, 2 females and 2 of undetermined sex. Gross examinations of dolphin carcasses recorded no evidence of human interaction, infectious disease or other significant pathology. Overall, 79% (11 of 14) of the

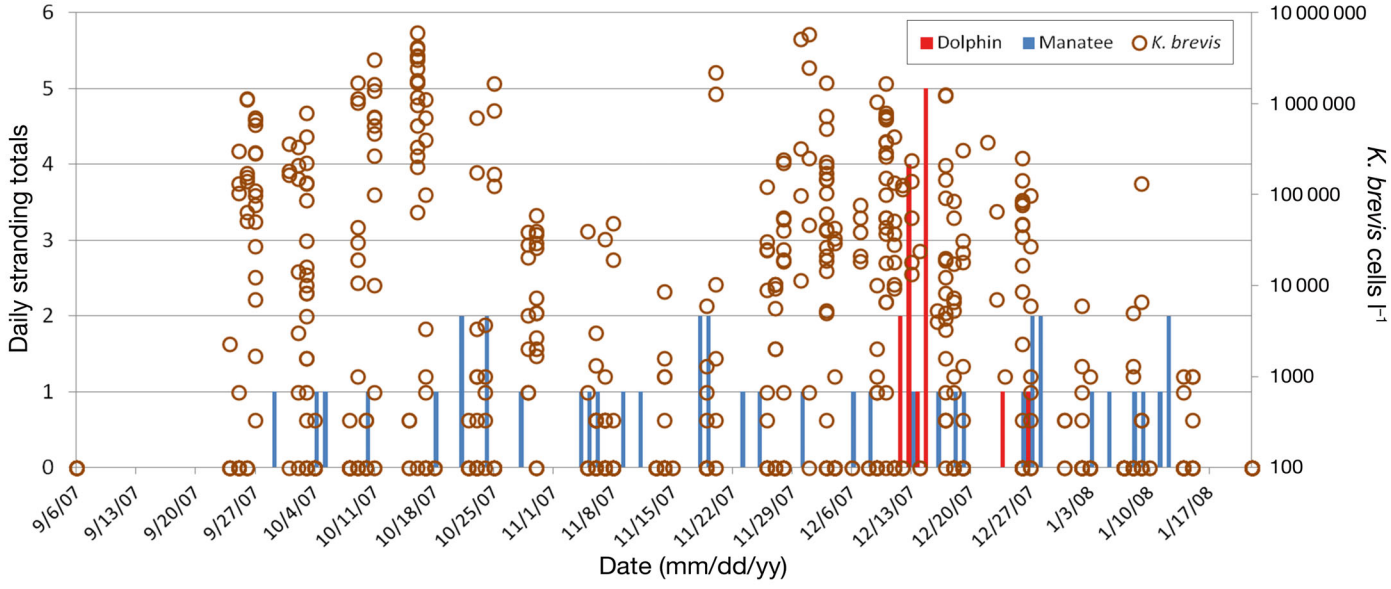

Fig. 2. Time series of *Karenia brevis* cell concentrations and dolphin and manatee strandings, Nassau County to St. Lucie County, Florida, USA

Table 1. Brevetoxin concentrations detected by enzyme-linked immunosorbent assays (ELISA) in bottlenose dolphin *Tursiops truncatus* samples (ng g^{-1} or ng ml^{-1}). <dl = below limit of detection. Dates are given as mm/dd/yy

Animal Field ID	Sex	Length (cm)	Stranding date	Stomach contents	Feces	Liver	Kidney	Muscle	Lung	Blood	Blubber	Spleen	Eye	Urine
Hubbs-0771-Tt	M	218	12/12/07	626		65[b]	35		21		16	27		
Hubbs-0772-Tt	F	222	12/12/07		557				22					
Hubbs-0773-Tt	M	229	12/12/07		454[b]	83	34	28	24	1	45	35	55	
Hubbs-0774-Tt	M	206	12/12/07	442	527	89	25	<dl	15	7	16	17	<dl	
Hubbs-0775-Tt	F	246	12/13/07		34	38	23	<dl	<dl	4	15			
Hubbs-0775-Tt-Fetus	M	42	12/13/07					13	6					
Hubbs-0776-Tt	M	201	12/13/07		270	77	25	7	15	6	<dl	11		
Hubbs-0777-Tt	U	182	12/14/07	39				<dl	8		42			
Hubbs-0778-Tt[a]	M	249	12/13/07							<dl				
Hubbs-0779-Tt[a]	M	265	12/13/07							<dl				
Hubbs-0780-Tt	M	240	12/15/07	170			56	17	17		52	37		
Hubbs-0781-Tt	U	243	12/15/07					16	50		72			
Hubbs-0782-Tt	M	253	12/24/07		<dl	<dl	<dl	<dl	<dl	<dl	<dl			<dl
Hubbs-0783-Tt	M	98	12/27/07			11	<dl	<dl	21		<dl		<dl	<dl
			N	4	6	7	8	10	12	7	10	5	3	2
			Min.	39	34	11	23	7	6	1	15	11		
			Max.	626	557	89	56	28	50	7	72	37		
			Mean	319	368	60	33	16	20	4	37	25	55	<dl

[a]Live animals sampled in Mosquito Lagoon
[b]Confirmed by liquid chromatography-mass spectrometry (LC-MS)

dolphins sampled tested positive for brevetoxin in at least 1 sample type (Table 1). For both live-rescue dolphins from the Mosquito Lagoon, only serum samples were available, and these were negative for brevetoxin. Excluding these, 92% (11 of 12) of the dead-stranded dolphins tested positive for brevetoxin. Gastrointestinal (GI) contents had the highest levels of brevetoxin, with concentrations up to 626 ng g^{-1} in brevetoxin-positive samples (4 of 4 positive stomach content samples, 39–626 ng g^{-1}; 5 of 6 for feces, 34–557 ng g^{-1}). With respect to distribution among tissue compartments, brevetoxin was detected in nearly all other sample types collected, including liver (8 of 9 samples testing positive, 11–89 ng g^{-1}), kidney (6 of 8, 23–56 ng g^{-1}), muscle (5 of 10, 7–28 ng g^{-1}), lung (10 of 12, 6–50 ng g^{-1}), blood (4 of 7, 1–7 ng ml^{-1}), blubber (7 of 10, 15–72 ng g^{-1}), spleen (5 of 5, 11–37 ng g^{-1}) and eye (1 of 2, 55 ng g^{-1}). Brevetoxin was not detected in the 2 urine samples collected. One whole fish (species not identified) was opportunistically sampled from the esophagus of Hubbs-0777-Tt and tested positive for brevetoxin concentrations of 40 and 754 ng g^{-1} in muscle and liver tissue, respectively (data not shown). Brevetoxins, both parent toxins and multiple metabolites, were also confirmed by LC-MS in samples from Hubbs-0771-Tt (liver, PbTx-3) and Hubbs-0773-Tt (feces, PbTx-3, PbTx-7, S-desoxybrevetoxin-B2, and brevetoxin-B2).

Manatee analyses

Based on total carcass length recorded at necropsy, the age and sex classes of the manatees sampled were distributed as follows: 11 adults (4 males, 7 females [of which 2 were pregnant]), 6 subadults (4 males, 2 females), and 16 calves (10 males, 6 females; Table 2). Cause of death was determined as watercraft trauma in 5 manatee carcasses. Ingestion of a fishing hook and line was determined as the cause of death in 1 carcass. Two calf carcasses were stillborn, and 1 calf had possibly been orphaned or abandoned. Three animals had died from cold stress, and 14 from other natural causes. None of the carcasses presented evidence of infectious disease or other significant pathology. Extreme decomposition prevented determination of cause of death in 7 carcasses. Of the 29 manatees (plus 1 fetus) sampled, 20 (69%) tested positive for brevetoxin in at least 1 sample type (Table 2), and for 11 of these brevetoxicosis was concluded to be the probable cause of death. Brevetoxicosis cases were defined as those with vascular congestion in one or more organs or tissues on necropsy, with brevetoxin concentrations detected in multiple samples and for which no other cause of death was apparent. Badly decomposed carcasses with no detectable gross findings were considered probable brevetoxicosis cases if they were brevetoxin-positive

Table 2. Brevetoxin concentrations detected by enzyme-linked immunosorbent assays (ELISA) in manatee *Trichechus manatus latirostris* samples (ng g^{-1} or ng ml^{-1}). <dl = below limit of detection. Dates are given as mm/dd/yy

Animal Field ID	Sex	Length (cm)	Stranding date	Stomach contents	Feces	Liver	Kidney	Muscle	Lung	Urine	Other
MSE0727	M	303	10/4/07								
MNE0736	M	150	10/5/07			<dl	<dl		<dl		
MEC0748	F	362	10/10/07	<dl		54					
MEC0749	F	109	10/18/07								
MEC0750	F	298	10/21/07	<dl		<dl			<dl		
MEC0750-fetus			10/21/07			<dl	<dl				
MEC0751	F	308	10/28/07	502				13			Tunicates, 210
MEC0752	M	307	11/4/07	<dl		<dl			<dl	1	
MEC0753	M	223	11/5/07	129		48			<dl	6	
MEC0754	M	236	11/6/07	84		52			<dl	5	Tunicates, 55
MEC0755	F	124	11/11/07			<dl			<dl	<dl	
MEC0756	F	240	11/18/07	<dl		9			<dl	<dl	
MEC0759	F	315	11/18/07	<dl		<dl	<dl		<dl		
MEC0758	F	145	11/19/07			<dl			<dl		
MEC0757	F	331	11/19/07	<dl		<dl			<dl	<dl	
MEC0760	F	242	11/23/07	1173		116			<dl	24	
MEC0761	M	138	11/25/07								
MEC0762	M	184	11/30/07	110				38	49		
MNE0739	M	168	12/8/07								
MEC0763	M	265	12/13/07	88		98	18		<dl	6	
MEC0764	M	319	12/16/07	106		71	13		11		
MEC0765	F	226	12/18/07	110		66	13		<dl		
MEC0766	F	342	12/19/07	85		85			<dl	8	Milk, 24
MEC0768	M	145	12/26/07			13	<dl		<dl		
MEC0767	F	216	12/27/07	7		57	17		7		
MEC0769	M	242	12/27/07		30	7	<dl		<dl		
MEC0771	F	307	12/28/07	<dl					<dl	<dl	
MEC0770	M	171	12/28/07	10		<dl			<dl	<dl	
MEC0801	M	221	1/3/08	<dl		7	<dl		<dl		
MEC0802	M	271	1/5/08	<dl	<dl	<dl	<dl		<dl		
MSE0801	M	241	1/9/08	394		74	21				
MEC0803	M	146	1/11/08	<dl		<dl	<dl		<dl		
MEC0805	F	146	1/12/08	12		12			<dl	<dl	
MEC0804	M	118	1/12/08		38	<dl	<dl		<dl		
			N	23	3	27	14	2	26	12	
			Min.	7	30	7	13	13	7	1	
			Max.	1173	38	116	21	38	49	24	
			Mean	216	34	51	16	26	22	9	

in multiple tissues and GI contents and at least one such sample contained brevetoxin at a concentration of at least 50 ng g^{-1}. As observed in the dolphins, GI contents had the highest levels of brevetoxin, with concentrations of 7–1173 ng g^{-1} in stomach contents (13 of 23 samples testing positive) and 30–38 ng g^{-1} in feces (2 of 3 samples positive). Brevetoxin was also detected in all other sample types, including liver (15 of 27, 7–116 ng g^{-1}), kidney (5 of 14, 13–21 ng g^{-1}), muscle (2 of 2, 13–38 ng g^{-1}), lung (3 of 26, 7–49 ng g^{-1}), and urine (6 of 12, 1–24 ng ml^{-1}). Milk opportunistically collected from one manatee (MEC0766) also contained measurable levels of brevetoxins (24 ng ml^{-1}). Stomach contents of all red tide-related carcasses contained seagrasses, indicating recent feeding, with samples of epifaunal tunicates (n = 2) collected from this seagrass material also testing positive for PbTx (Table 2).

DISCUSSION

Although *Karenia brevis* is often present at background concentrations in seawater and brevetoxin has been detected at low levels in marine mammals along the Atlantic coast of Florida (S. E. Fire unpubl. data), *K. brevis* blooms are relatively infrequent and marine mammal mortality events associated with

brevetoxin exposure have not previously been reported in this region (Tester & Steidinger 1997). A 1987–1988 mass mortality of bottlenose dolphins between Florida and New Jersey was initially thought to have been caused by an expatriate *K. brevis* bloom from the Gulf of Mexico (Geraci 1989), but due to a lack of adequate brevetoxin analytical methods and the presence of co-occurring morbillivirus infection, the involvement of brevetoxin remains speculative (Duignan et al. 1996, Van Dolah 2005). In this case, however, the analytical data support the connection. The severity of the bloom (with numerous samples across 6 counties exceeding 1 000 000 cells l^{-1} between September and December 2007; Fig. 1) was also similar to that of previous blooms associated with *K. brevis*-related mass mortalities along the Gulf of Mexico coast (Landsberg & Steidinger 1998, FWC 2008, Twiner et al. 2012). Negative effects of the bloom extended to other natural resources as well, including fish kills (131 reports of red tide-related fish kills were received by the FWC Fish Kill Hotline between September and December 2007; FWC unpubl. data) and prolonged closures of shellfish harvest areas in 4 counties to prevent Neurotoxic Shellfish Poisoning.

The total number of dolphins stranding during this study was fairly modest compared with similar Florida mortality events in which more than 100 dolphins died (Mase et al. 2000, Flewelling et al. 2005, Twiner et al. 2012). However, compared with historical stranding records, the number of dolphins that stranded during this event (n = 14) was several times greater than the mean number of strandings (mean = 3.5) for December over an 11 yr period of consistent response effort (Fig. 3a). Manatee mortality did not reach sufficient numbers to initiate official notification of a possible unusual mortality event, which is greater than 7 manatee carcasses or distressed manatees found in a localized area within 72 h (USFWS 1997). However, compared with historical stranding records for Volusia County to St. Lucie County, the number of manatees that stranded in this event (n = 33) was also notably greater than the mean number of strandings (mean = 20) for September to January over the previous 10 yr of consistent response effort (Fig. 3b).

Both the degree of accumulation and distribution of brevetoxin in tissues and fluids collected from the dolphins and manatees were similar to what has been reported for previous *K. brevis*-associated mortality events elsewhere in Florida (Fig. 4) (Mase et al. 2000, Flewelling et al. 2005). Compared with other types of samples, gastrointestinal contents had the highest concentrations of brevetoxin in each individual sampled (max. 626 ng g^{-1} dolphin, 1173 ng g^{-1} manatee), typically followed by liver (max. 89 ng g^{-1} dolphin, 116 ng g^{-1} manatee) and kidney (max. 56 ng g^{-1} dolphin, 21 ng g^{-1} manatee). Where similar sample types were collected and could be compared across events, brevetoxin exposure in the present study was comparable to that seen in conjunction with dolphin die-offs along the Gulf of Mexico coast of Florida in 1999–2000, 2004 and 2005–2006, and with a manatee die-off in 2002 (Fig. 4). Finfish and seagrass were observed in stomach contents of the dolphins and manatees, respectively, and high concentrations of brevetoxin were detected in the fish and epifaunal tunicates from these stomach content samples. This reinforces previous studies that have identified these dietary items as brevetoxin vectors, which, when consumed, lead to an accumulation of toxins in manatees and dolphins (Flewelling et al. 2004, Naar et al. 2007, Fire et al. 2008). Also of note was the general lack of brevetoxin detected in the

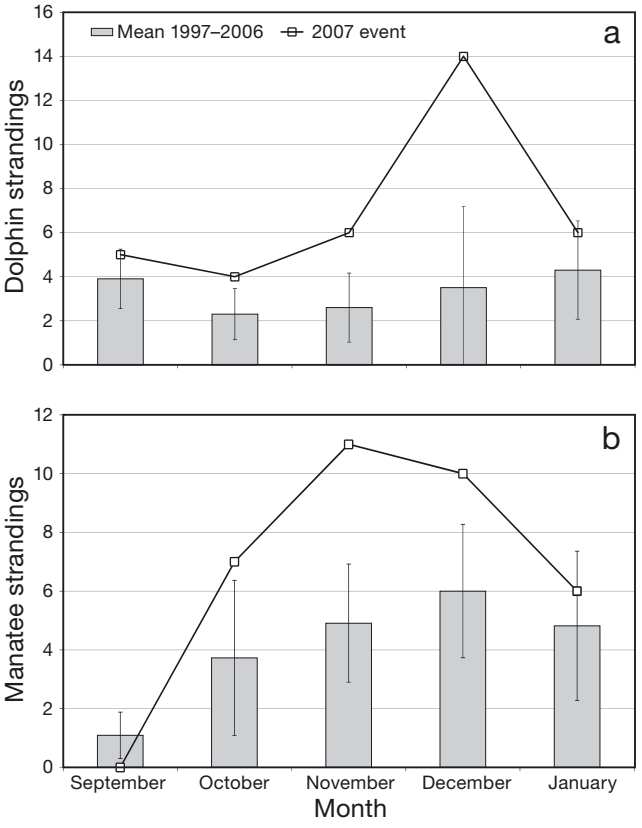

Fig. 3. Historical mean (a) dolphin and (b) manatee strandings for September to January 1997–2006 in Volusia, Brevard, Indian River, and St. Lucie counties, Florida, USA, compared to the 2007 event documented in this study (see Fig. 1 for locations of water bodies)

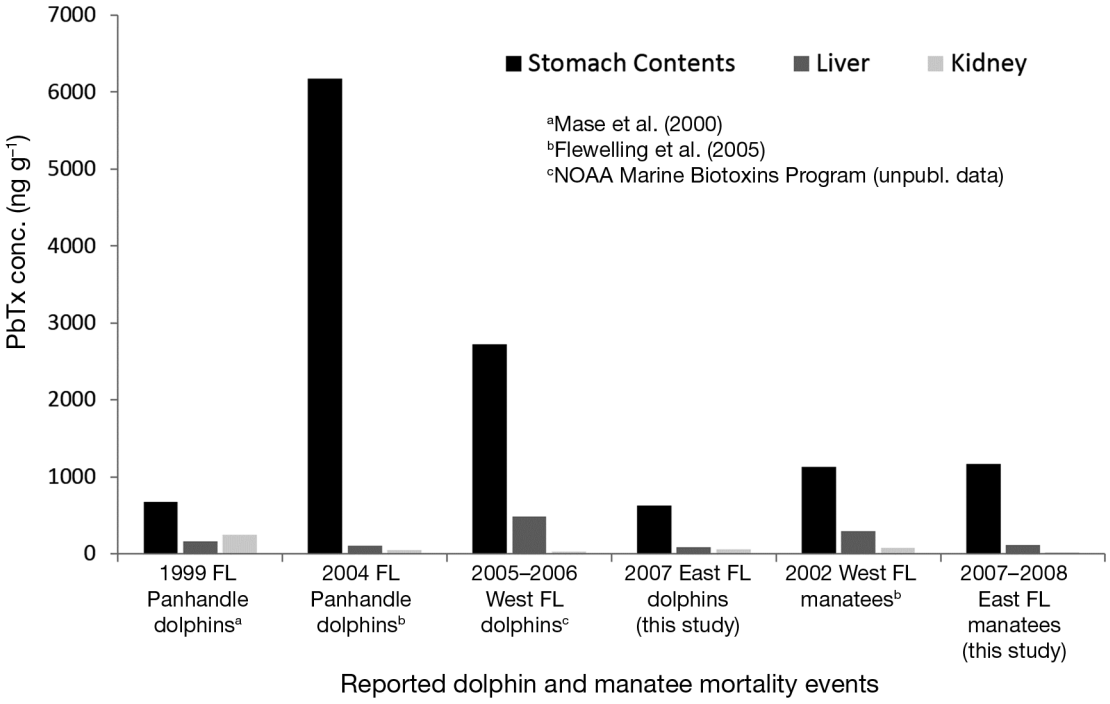

Fig. 4. Mean concentration of brevetoxins in selected sample types from dolphins and manatees: a comparison to previous mortality events

manatee lung samples, with only 3 of 26 samples testing positive. Although brevetoxins can become aerosolized and enter the respiratory tract (Pierce et al. 1990, Bossart et al. 1998), the concentrations detected in these few samples were relatively low (7–49 ng g^{-1}). Together with the high brevetoxin levels detected in stomach contents, these data suggest dietary exposure rather than inhalation of brevetoxin as the primary route of intoxication in manatees during *K. brevis* blooms. In the absence of other evidence of possible causes of death in these animals from gross pathology (human interaction, infectious disease, etc.), and in light of the evidence of brevetoxin exposure in these animals during the 2007 *K. brevis* bloom, brevetoxicosis is the presumed cause of this mortality event.

Our findings also provide new evidence that marine mammals are exposed to brevetoxin in early developmental stages. Brevetoxins were detected in milk from a lactating manatee (MEC0766) as well as in a dolphin fetus (Hubbs-0775-Tt-Fetus) and a dolphin neonate (Hubbs-0783-Tt, Table 1). The presence of brevetoxin in the dolphin fetus indicates *in utero* exposure during the *K. brevis* bloom; its presence in neonate tissues suggests *in utero* exposure and/or maternal transfer of brevetoxin through lactation, since animals of this age are nursing calves and do not forage independently (Wells et al. 1987). These results confirm in the field what has been demonstrated experimentally in rodents: that placental transport of brevetoxin or its metabolites occurs following maternal exposure (Benson et al. 2006). This finding has serious implications for conservation efforts that protect dolphin and manatee health, since other HAB toxins are known to cause permanent neurological damage to marine mammals exposed to HAB toxins as perinates. For example, the HAB toxin domoic acid, an analog of the neurotransmitter glutamate, can cause reproductive failure and a chronic epileptic syndrome in California sea lions *Zalophus californianus* exposed to the toxin *in utero* or soon after birth via toxin-contaminated milk (Brodie et al. 2006, Goldstein et al. 2008). In addition, both brevetoxins and the structurally related ciguatoxins caused embryo toxicity and developmental abnormalities in experimentally dosed finfish (Kimm-Brinson & Ramsdell 2001, Colman et al. 2004). If brevetoxins are similarly able to induce neurological changes at these critical stages in dolphin and manatee development, then the health of these protected species may be threatened at the individual as well as the population level.

In conclusion, we describe here a novel brevetoxin-associated multi-species mortality event along the east coast of Florida, with evidence of brevetoxin intoxication across all age and sex classes. We further

show that red tides on Florida's east coast, though rare, can result in mass mortality events with levels of brevetoxin exposure in dolphins and manatees similar to those reported elsewhere in Florida. We also demonstrate the first incidence of maternal transfer of brevetoxins in free-ranging marine mammals in their natural habitat during a *K. brevis* bloom. These findings support current management policies under which phytoplankton and marine mammals are biomonitored nationwide in efforts to assess the impacts of HABs and their associated toxins on protected marine species, ultimately informing marine policy decisions on conservation of sentinel species such as bottlenose dolphins and manatees in US coastal waters.

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LITERATURE CITED

Balmer BC, Schwacke LH, Wells RS, George RC and others (2011) Relationship between persistent organic pollutants (POPs) and ranging patterns in common bottlenose dolphins (*Tursiops truncatus*) from coastal Georgia, USA. Sci Total Environ 409:2094–2101

Benson JM, Gomez AP, Statom GL, Tibbetts BM and others (2006) Placental transport of brevetoxin-3 in CD-1 mice. Toxicon 48:1018–1026

Bonde RK, Aguirre AA, Powell J (2004) Manatees as sentinels of marine ecosystem health: are they the 2000-pound canaries? EcoHealth 1:255–262

Bossart GD (2006) Marine mammals as sentinel species for oceans and human health. Oceanography (Wash DC) 19: 134–137

Bossart GD, Baden DG, Ewing RY, Roberts B, Wright SD (1998) Brevetoxicosis in manatees (*Trichechus manatus latirostris*) from the 1996 epizootic: gross, histologic, and immunohistochemical features. Toxicol Pathol 26: 276–282

Brodie EC, Gulland FMD, Greig DJ, Hunter M and others (2006) Domoic acid causes reproductive failure in California sea lions (*Zalophus californianus*). Mar Mamm Sci 22:700–707

Colman JR, Bottein Dechraoui MY, Dickey RW, Ramsdell JS (2004) Characterization of the developmental toxicity of Caribbean ciguatoxins in finfish embryos. Toxicon 44: 59–66

Duignan PJ, House C, Odell DK, Wells RS and others (1996) Morbillivirus infection in bottlenose dolphins: evidence for recurrent epizootics in the western Atlantic and Gulf of Mexico. Mar Mamm Sci 12:499–515

Fauquier DA, Fire S, Flewelling L, Pierce R and others (2005) Brevetoxin-induced morbidity and mortality in stranded and wild dolphins from central west Florida. Final Technical Report to Harbor Branch Oceanographic Institution's PWD Program, Ft. Pierce, FL

Fire SE, Van Dolah FM (2012) Marine biotoxins: emergence of harmful algal blooms as health threats to marine wildlife. In: Aguirre AA, Ostfeld RS, Daszak P (eds) New directions in conservation medicine: applied cases of ecological health. Oxford University Press, New York, NY, p 374–389

Fire SE, Flewelling LJ, Naar J, Twiner MJ and others (2008) Prevalence of brevetoxins in prey fish of bottlenose dolphins in Sarasota Bay, Florida. Mar Ecol Prog Ser 368: 283–294

Fire SE, Wang Z, Byrd M, Whitehead HR, Paternoster J, Morton SL (2011) Co-occurrence of multiple classes of harmful algal toxins in bottlenose dolphins (*Tursiops truncatus*) stranding during an unusual mortality event in Texas; USA. Harmful Algae 10:330–336

Flewelling LJ, Abbott JP, Hammond DG, Landsberg J and others (2004) Seagrass as a route of brevetoxin exposure in the 2002 red tide-related manatee mortality event [abstract]. Harmful Algae 3:205

Flewelling LJ, Naar JP, Abbott JP, Baden DG and others (2005) Red tides and marine mammal mortalities. Nature 435:755–756

FWC (Florida Fish and Wildlife Commission) (2008) Florida manatee (*Trichechus manatus latirostris*) red tide mortality event in southwestern Florida March–April 2003. Final Report to the Working Group on Marine Mammal Unusual Mortality Events

Geraci JR (1989) Clinical investigation of the 1987-88 mass mortality of bottlenose dolphins along the US central and south Atlantic coast. Final report to the National Marine Fisheries Service, US Navy Office of Naval Research, and Marine Mammal Commission. Ontario Veterinary College, University of Guelph

Geraci JR, Lounsbury VJ (2005) Marine mammals ashore: a field guide for strandings, 2nd edn. National Aquarium in Baltimore, Baltimore, MD

Goldstein T, Mazet JAK, Zabka TS, Langlois G and others (2008) Novel symptomatology and changing epidemiology of domoic acid toxicosis in California sea lions (*Zalophus californianus*): an increasing risk to marine mammal health. Proc R Soc B 275:267–276

Gunter G, Williams RH, Davis CC, Smith FGW (1948) Catastrophic mass mortality of marine animals and coincident phytoplankton bloom on the west coast of Florida, November 1946 to August 1947. Ecol Monogr 18: 309–324

Heil CA, Steidinger KA (2009) Monitoring, management, and mitigation of *Karenia* blooms in the eastern Gulf of Mexico. Harmful Algae 8:611–617

Kimm-Brinson KL, Ramsdell JS (2001) The red tide toxin, brevetoxin, induces embryo toxicity and developmental abnormalities. Environ Health Perspect 109:377–381

Laist DW, Knowlton AR, Mead JG, Collet AS, Podesta M (2001) Collisions between ships and whales. Mar Mamm Sci 17:35–75

Landsberg JH, Steidinger KA (1998) A historical review of *Gymnodinium breve* red tides implicated in mass mortalities of the manatee (*Trichechus manatus latirostris*) in Florida, USA. In: Reguera B, Blanco J, Fernandez ML, Wyatt T (eds) Proc 8th Int Conf Harmful Algae. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO, Vigo, Spain, p 97–100

Landsberg J, Van Dolah F, Doucette G (2005) Marine and estuarine harmful algal blooms: impacts on human and animal health. In: Belkin S, Colwell RR (eds) Oceans and health: pathogens in the marine environment. Springer, New York, NY, p 165–215

Landsberg JH, Flewelling LJ, Naar J (2009) *Karenia brevis* red tides, brevetoxins in the food web, and impacts on natural resources: decadal advancements. Harmful Algae 8:598–607

Mase B, Jones W, Ewing R, Bossart G and others (2000) Epizootic in bottlenose dolphins in the Florida panhandle: 1999–2000. In: Baer CK (ed) Proc Am Assoc Zoo Veterans and Int Assoc Aquat Anim Med, p 522–525

Naar J, Bourdelais A, Tomas C, Kubanek J and others (2002) A competitive ELISA to detect brevetoxins from *Karenia brevis* (formerly *Gymnodinium breve*) in seawater, shellfish, and mammalian body fluid. Environ Health Perspect 110:179–185

Naar JP, Flewelling LJ, Lenzi A, Abbott JP and others (2007) Brevetoxins, like ciguatoxins, are potent ichthyotoxic neurotoxins that accumulate in fish. Toxicon 50:707–723

NMFS (National Marine Fisheries Service) (2004) Interim report on the bottlenose dolphin (*Tursiops truncatus*) unusual mortality event along the Panhandle of Florida, March-April 2004

NOAA (National Oceanic and Atmospheric Administration) (2013) U.S. Atlantic and Gulf of Mexico Marine Mammal Stock Assessments – 2013. NOAA Technical Memorandum NMFS-NE-228

O'Shea T, Langtimm C (1995) Estimation of survival of adult Florida manatees in the Crystal River, at Blue Spring, and on the Atlantic Coast. In: O'Shea T, Ackerman B, Perciva H (eds) Population biology of the Florida manatee, US Department of the Interior, National Biological Service, Information and Technology Report, p 194–222

O'Shea TJ, Moore JF, Kochman HI (1984) Contaminant concentrations in manatees in Florida. J Wildl Manag 48: 741–748

Pierce RH, Henry MS, Proffitt LS, Hasbrouck PA (1990) Red tide toxin (brevetoxin) enrichment in marine aerosol. In: Graneli E, Sundstrom B, Elder L, Anderson DM (eds) Toxic marine phytoplankton. Elsevier, New York, NY, p 397–402

Read AJ, Drinker P, Northridge S (2006) Bycatch of marine mammals in U.S. and global fisheries. Conserv Biol 20: 163–169

Steidinger KA, Vargo GA, Tester PA, Tomas CR (1998) Bloom dynamics and physiology of *Gymnodinium breve* with emphasis on the Gulf of Mexico. In: Anderson DM, Cembella AD, Hallegraeff GM (eds) Physiological ecology of harmful algal blooms. Springer-Verlag, Berlin, p 133–153

Tester PA, Steidinger KA (1997) *Gymnodinium breve* red tide blooms: initiation, transport, and consequences of surface circulation. Limnol Oceanogr 42:1039–1051

Twiner MJ, Flewelling LJ, Fire SE, Bowen-Stevens SR and others (2012) Comparative analysis of three brevetoxin-associated bottlenose dolphin (*Tursiops truncatus*) mortality events in the Florida Panhandle region (USA). PLoS ONE 7:e42974

USFWS (US Fish and Wildlife Service) (1997) Contingency plan for catastrophic manatee rescue and mortality events. Prepared by the Manatee Recovery Program, Jacksonville, FL, Field Office for Southeast Region USFWS, Atlanta, GA

USFWS (US Fish and Wildlife Service) (2009) West Indian Manatee (*Trichechus manatus*) Florida Stock (Florida subspecies, *Trichechus manatus latirostris*). US Fish and Wildlife Service, Jacksonville, FL

Van Dolah FM (2005) Effects of harmful algal blooms. In: Reynolds J, Perrin W, Reeves R, Montgomery S, Ragen T (eds) Marine mammal research: conservation beyond crisis. Johns Hopkins University Press, Baltimore, MD, p 85–101

Wells RS, Scott MD, Irvine AB (1987) The social structure of free-ranging bottlenose dolphins. In: Genoways HH (ed) Current mammalogy, Book 1. Plenum Press, New York, NY, p 247–305

Wells RS, Rhinehart HL, Hansen LJ, Sweeney JC and others (2004) Bottlenose dolphins as marine ecosystem sentinels: developing a health monitoring system. EcoHealth 1: 246–254

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