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BREVETOXIN IN BLOOD, BIOLOGICAL FLUIDS, AND TISSUES OF SEA TURTLES NATURALLY EXPOSED TO *KARENIA BREVIS* BLOOMS IN CENTRAL WEST FLORIDA

Deborah A. Fauquier, D.V.M., M.P.V.M., Leanne J. Flewelling, Ph.D., Jennifer Maucher, M.S., Charles A. Manire, D.V.M., Victoria Socha, B.S., Michael J. Kinsel, D.V.M., Dipl. A.C.V.P., Brian A. Stacy, D.V.M., Ph.D., Dipl. A.C.V.P., Michael Henry, M.S., Janet Gannon, M.S.N.R., John S. Ramsdell, Ph.D., and Jan H. Landsberg, Ph.D.

Abstract: In 2005 and 2006, the central west Florida coast experienced two intense *Karenia brevis* red tide events lasting from February 2005 through December 2005 and August 2006 through December 2006. Strandings of sea turtles were increased in the study area with 318 turtles ($n = 174$, 2005; $n = 144$, 2006) stranding between 1 January 2005 and 31 December 2006 compared to the 12-yr average of 43 ± 23 turtles. Live turtles ($n = 61$) admitted for rehabilitation showed clinical signs including unresponsiveness, paresis, and circling. Testing of biological fluids and tissues for the presence of brevetoxin activity by enzyme-linked immunosorbent assay found toxin present in 93% (52 of 56) of live stranded sea turtles, and 98% (42 of 43) of dead stranded sea turtles tested. Serial plasma samples were taken from several live sea turtles during rehabilitation and toxin was cleared from the blood within 5–80 days postadmit depending upon the species tested. Among dead animals the highest brevetoxin levels were found in feces, stomach contents, and liver. The lack of significant pathological findings in the majority of animals necropsied supports toxin-related mortality.

Key words: *Caretta caretta*, harmful algal bloom, *Karenia brevis*, red tide, sea turtle, toxin.

INTRODUCTION

Harmful algal blooms are suggested to have caused or contributed to mortality events documented in marine mammals, sea turtles, and sea birds throughout the world's oceans.[14,18,29,32,35] In Florida waters, the most prevalent harmful algae is the dinoflagellate *Karenia brevis,* which produces potent neurotoxins called brevetoxins. High mortality due to brevetoxicosis from *K. brevis* has been documented in fish and manatees along the western coast of Florida.[1,13,16] Significant increases in mortality of dolphins, manatees, and sea birds have been documented during red tide events with recent evidence implicating brevetoxin involvement.[6,11,17,19]

Severe and prolonged *K. br*evis bloom events occurred off the west coast of Florida from February through December 2005 and from August through December 2006,[12] with *K. brevis* cell concentrations reaching levels of 100 million cells per liter of seawater. As point of reference, concentrations of 1,000 cells per liter or less are considered background levels, and 100,000 cells per liter can cause fish kills and respiratory irritation in humans.[30] During the 2005 and 2006 *K. brevis* blooms, sea turtle strandings in the study area documented by Mote Marine Laboratory (MML) increased threefold in 2005 ($n = 174$) and twofold in 2006 ($n = 144$) over the previous 12-yr average (43 ± 23 turtles/yr) of turtles stranding in Mote's stranding response range (Fauquier, unpubl. data).

Karenia brevis blooms are frequent events in southwest Florida but the overall impact on wildlife populations has previously been difficult to assess due to the lack of appropriate methods for quantifying brevetoxin in tissues. The development of a competitive enzyme-linked immunosorbent assay (ELISA)[22,23] has facilitated the assessment of exposure and determination of brevetoxin concentrations in biological fluids and tissues in stranded manatees, dolphins, sharks, and birds in Florida.[5,6,10,11,31]

From Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, Florida 34236, USA (Fauquier, Gannon, Henry, Manire, Socha); Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, 100 8th Avenue S.E., St. Petersburg, Florida 33701, USA (Flewelling, Landsberg); National Ocean Service, Center for Coastal Environmental Health and Biomolecular Research, 219 Fort Johnson Road, Charleston, South Carolina 29412, USA (Maucher, Ramsdell); University of Illinois, Zoological Pathology Program, 2001 S. Lincoln, Maywood, Illinois 60153, USA (Kinsel); and University of Florida, College of Veterinary Medicine, Large Animal Clinical Sciences, 2015 S.W. 16th Avenue, Gainesville, Florida 32610, USA (Stacy). Present address (Manire): Loggerhead Marinelife Center, 14200 U.S. Highway One, Juno Beach, Florida 33408, USA. Correspondence should be directed to Dr. Fauquier (fauquierd@gmail.com).

Although all species of sea turtles in U.S. waters are listed on the Endangered Species Act of 1973 as endangered or threatened, limited data have been available linking brevetoxin exposure to sea turtle morbidity and mortality.[19] As long-lived animals, sea turtles can be indicator species alerting scientists to new and emerging diseases affecting wild populations.

In this study the presence and clearance of brevetoxin from biological fluids and tissues of stranded sea turtles naturally exposed to brevetoxin was monitored during *K. brevis* blooms. In addition, the spectrum of gross and histologic lesions associated with the presence of brevetoxin in tissues from stranded sea turtles was investigated.

MATERIAL AND METHODS

Study population and area

Live and dead animals consisted of stranded sea turtles that were recovered and brought into rehabilitation or necropsied at MML from 1 January 2005 through 31 December 2006. The study area ranged from Pinellas County in the north (28°8′59″N, 82°49′30″W) to Collier County in the south (25°44′45″N, 81°25′11″W) with animals chiefly recovered from Sarasota County (Fig. 1). The main species recovered were loggerhead (*Caretta caretta*), Kemp's ridley (*Lepidochelys kempii*), and green (*Chelonia mydas*) sea turtles. Blood samples were collected from most live animals on admission to MML's Sea Turtle Rehabilitation Hospital. Additionally, serial blood samples (1–3 ml) were collected from a select number of live sea turtles at admission and opportunistically thereafter, ranging from every 2 days to every 2 wk, until clinical signs resolved.

Dead animals, including animals recovered dead and those that died during rehabilitation, were necropsied according to techniques described by Wyneken.[36] Body condition and sex were determined at necropsy. Representative samples of all organ systems including but not limited to brain, heart, intestine, kidney, liver, lung, spleen, and stomach from fresh carcasses were collected for histopathology to establish a cause of death as part of a comprehensive postmortem evaluation. All tissue samples for histopathology were fixed in 10% neutral buffered formalin and submitted to the University of Illinois Zoological Pathology Program or to the University of Florida for examination. Samples collected for brevetoxin analysis included bile, feces, kidney, liver, lung, and stomach contents. In a few animals intestinal fluids and urine were also collected. These tissues were frozen at –20°C until analyzed.

Karenia brevis cell densities from two fixed reference stations located in Sarasota Bay (New Pass channel and City Island Seagrass Flats; Fig. 1) were obtained from MML's and the Florida Fish and Wildlife Conservation Commission's Fish and Wildlife Research Institute's Red Tide Monitoring Program.[12] Surface water samples were collected one to five times per week and immediately preserved in Utermohl's solution[15] and stored in the dark at room temperature. Enumeration of *K. brevis* abundance was conducted using an inverted Olympus CK40 microscope. *Karenia brevis* bloom conditions were defined as cell densities $\geq 1 \times 10^5$ cells/L anywhere in the study area within a 30-day period.

Laboratory analysis

A competitive ELISA (MARBIONC, Wilmington, North Carolina 28403, USA) was used to detect brevetoxins in turtle tissues and biological fluids.[9, 22] To prepare samples for analysis, tissues were extracted twice in 80% aqueous methanol (1:5 w/v). The pooled extracts were partitioned with hexane, and the methanol fraction was retained for analysis. Bile was extracted by applying 0.5 ml to a preconditioned C18 solid phase extraction column (Supelco Bellefonte, Pennsylvania 16823 USA, 500 mg, 3 ml). The column was washed with 25% methanol and toxins were eluted with 100% methanol. Urine and serum–plasma samples were centrifuged to clarify (if necessary) and analyzed without extraction. The ELISA recognizes all congeners and metabolites of brevetoxin that have a brevetoxin-B–type backbone, which is the dominant type of toxin (>80% of toxins present); congeners and metabolites of brevetoxin that have a brevetoxin-A–type backbone are recognized by this assay but at much lower affinities.[9] Standard curves were generated using brevetoxin-3 (PbTx-3). Results are expressed in nanograms per gram as PbTx-3 equivalents (ng PbTx-3 eq./g) and reflect the overall concentration of brevetoxins and brevetoxin-like compounds in the sample. The detection limit of the assay was approximately 5.0 ng PbTx-3 eq/g of tissue, 2.0 ng PbTx-3 eq/ml for bile, and 1.0 ng PbTx-3 eq/ml for plasma and urine.

Additionally, whole blood from live animals was analyzed for brevetoxin using a direct competitive ELISA optimized for blood collection cards.[22] Briefly, dried blood samples (~100 μl)

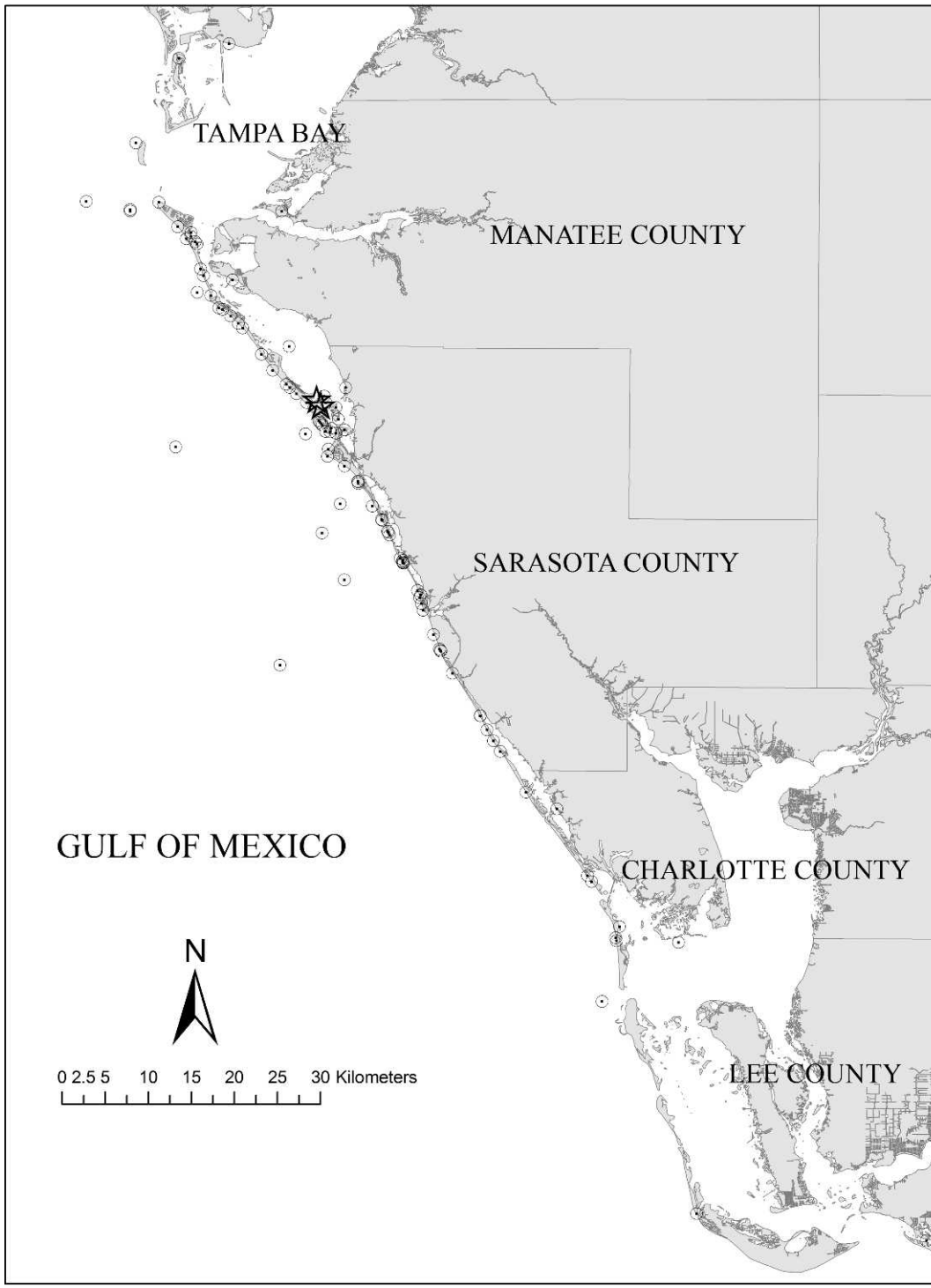

Figure 1. Map of the study area in central west Florida. Circles with dots represent locations of stranded sea turtles ($n = 99$) tested for brevetoxin during the entire study period 2005–2006; stars represent locations of *Karenia brevis* cell count reference stations.

were eluted from blood cards with phosphate-buffered saline with 6% methanol, followed by 100% acetonitrile to precipitate proteins. The brevetoxin antibody is specific to ring one of type B brevetoxins and was previously characterized by radioimmunoassay.[27] The detection limit of the assay was ≥1.0 ng PbTx-3 eq/ml of blood.

Since "brevetoxin" is a suite of at least 14 closely related toxic compounds, the potential exists for several individual toxins to be present in the same sample. To confirm the presence of brevetoxins, high performance liquid chromatography with mass spectrometry (LC-MS) analysis was performed on a subset of samples with high concentrations of brevetoxin (>200 ng/g) as demonstrated by ELISA. Prior to LC-MS analyses, a 1-g equivalent of extract was diluted to 25% methanol and passed through a preconditioned Strata-X cartridge (Phenomenex®, Torrance, California 90501, USA; 60 mg, 3 ml). The cartridge was washed with 9 ml of 25% methanol, and toxins were eluted with 5 ml of 100% methanol. The methanol extract was then evaporated to dryness and redissolved in 1 ml of 100% methanol. Analyses were performed on a Thermo-Finnigan AqA HPLC/MS with SpectraSystems (Thermo Fisher Scientific, West Palm Beach, Florida 33407, USA); LC Pump P4000, Autosampler AS3000, and a Degasser SCM1000 (Thermo Fisher Scientific); scanned from 204-1216 atomic mass unit (AMU) with a scan rate of 1.1 scans/sec; a Phenomenex Security Guard C-18 guard column with a Phenomenex® Luna C-18 5Fm 250 × 2mm analytical column (Phenomenex), and solvent gradient composed of acidified (0.3% acetic acid) binary gradient of acetonitrile and water (ACN/H_2O) with initial conditions starting at 50:50 ACN/H_2O and increasing to 95:5 ACN/H_2O over 40 min. Parent brevetoxins (PbTx-1:867, PbTx-2:895, PbTx-3:897, PbTx-6:911, PbTx-7:869, PbTx-9:899, PbTx-10:871, brevanal:657) and brevetoxin metabolites (Cyst-PbTx-B:1018, Ox-Cyst-PbTx-B:1034, Cyst-PbTx-A:990, and Ox-Cyst-PbTx-A:1006) were monitored at indicated masses. The instrument was calibrated with a standard brevetoxin mix containing PbTx-2 and PbTx-3 obtained from the Center for Marine Science, University of North Carolina Wilmington, North Carolina 28403, USA.

Statistical analysis

Agreement between the two ELISA testing methods was compared by using the Kappa test.[8] Differences between brevetoxin concentrations in biological fluids and tissue samples for both live stranded animals that were released or died, as well as dead stranded animals were analyzed using the Kruskal-Wallis test, all values had nonnormal distributions.[37] For all statistical tests, results were considered significant at $P \leq 0.05$. All statistical calculations and geographic information system mapping were performed using Minitab 15 (Minitab Inc., State College, Pennsylvania, 16801, USA), MedCalc 12.3.0 (MedCalc Software, Gent, 9030, Belgium), or ArcInfo 9.2 (ESRI, Redlands, California, 92373, USA) software, or some combination of these.

RESULTS

From 1 January 2005 to 31 December 2006, there were 318 sea turtle strandings in MML's stranding area, which represented a two- to threefold increased in strandings (Fig. 2). During this period 255 animals were recovered dead, and 63 animals were recovered live with two live animals released at the stranding site and not brought into rehabilitation. There were 204 loggerhead turtles, 65 Kemp's ridley turtles, 36 green turtles, 4 hawksbill turtles (*Eretmochelys imbricata*), 2 leatherback turtles (*Dermochelys coriacea*), and 7 turtles of undetermined species recovered.

The study area experienced a bloom of *K. brevis* from February through December 2005 and from August through December 2006 (Fig. 2), with high counts (>100,000 cells/L) occurring in 2005 from mid-January to mid-March and again from late May to mid-October. In 2006, high counts occurred from August through November.

A total of 99 sea turtles were tested for brevetoxin over the study period, with 62 animals tested in 2005 and 37 in 2006. There were 59 loggerhead, 26 Kemp's ridley, and 14 green turtles tested. Of these animals 48 were female, 28 were male, and 23 were of undetermined gender. Stage of maturity was estimated by straight carapace length or determined by examination of gonads at necropsy and there were 18 adult and 81 subadult turtles examined (all adults were loggerheads). Peak strandings occurred in the summer of 2005 and summer and fall of 2006.

During the *K. brevis* blooms some live turtles admitted for rehabilitation had clinical neurologic signs including head bobbing, muscle twitching, and jerky body movements. In addition, several animals had more severe signs, including generalized lethargy and unresponsiveness. All turtles were removed from the environment that contained the toxins, placed in water free of the

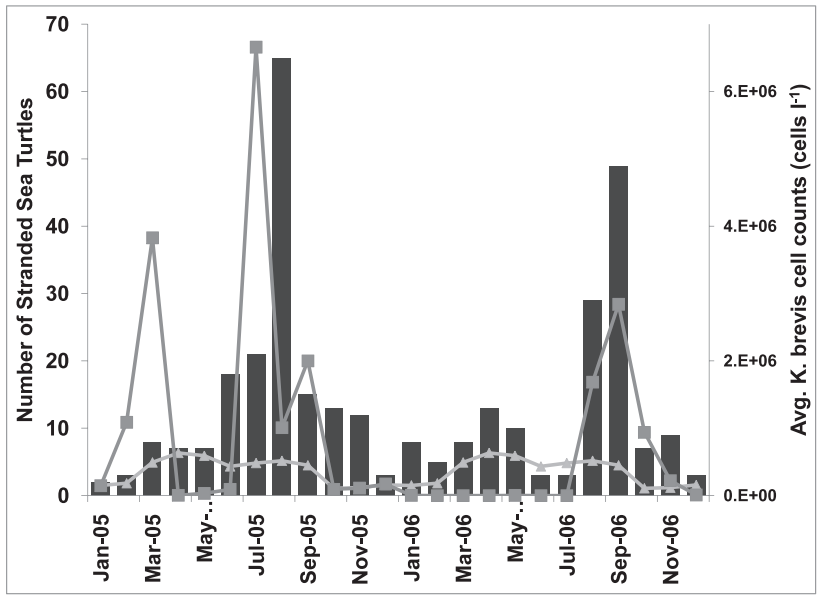

Figure 2. Total number of stranded sea turtles by month ($n = 318$). Black bars represent monthly turtle strandings; grey squares represent average monthly *Karenia brevis* cell counts (cells/L) at New Pass, Sarasota Bay; light grey triangles represent 12-yr monthly average sea turtle strandings.

toxins, and treated with supportive care including gavage feeding and subcutaneous fluids.[21]

A total of 56 live sea turtles were tested, 50 premortem and 6 postmortem; all postmortem tested animals were positive for brevetoxin. Premortem testing of blood and biological fluids for the presence of brevetoxin found brevetoxin activity in at least one sample in 92% (46 of 50) of live stranded sea turtles (Table 1). The highest brevetoxin concentrations at admission for all species ranged from below the level of detection (<ld) to 107 ng PbTx-3 eq/ml in plasma ($n = 45$), and 30 to 61,078 ng PbTx-3 eq/g in feces ($n = 12$). The mean brevetoxin value for plasma was 31 ng PbTx-3 eq/ml and for whole blood was 23 ng PbTx-3 eq/ml. There was agreement between the two ELISA testing methods in plasma and whole blood ($n = 29$, $k = 0.711$, Kappa test). Sea turtles

Table 1. Premortem brevetoxin concentrations (ng brevetoxin-3 eq/g) measured in plasma, whole blood, urine, and feces from live sea turtles stranding of the coast of central west Florida during *Karenia brevis* red tide conditions in 2005 and 2006 ($n = 50$).[a]

Species	Day sample taken	+/*N*	Mean	Median	Range
Plasma					
Green	0–1	3/6	1.2	0.5	<ld–4
Kemp's ridley	0–1	4/5	63	78	<ld–82
Loggerhead	0–1	29/34	32	21	<ld–107
Whole blood					
Green	0–1	3/6	1.6	1.0	<ld–4
Kemp's ridley	0–1	4/5	36	35	<ld–82
Loggerhead	0–1	26/33	25	13	<ld–81
Urine					
Kemp's ridley	1	1/1	7.4		
Loggerhead	1	1/1	1.7		
Feces					
Kemp's ridley	0–1	4/4	679	725	390–877
Loggerhead	0–10	8/8	8,398	258	30–61,078

[a] +/*N* = number of positive animals out of total animals tested. <ld = below level of detection, some animals had only one fluid type tested.

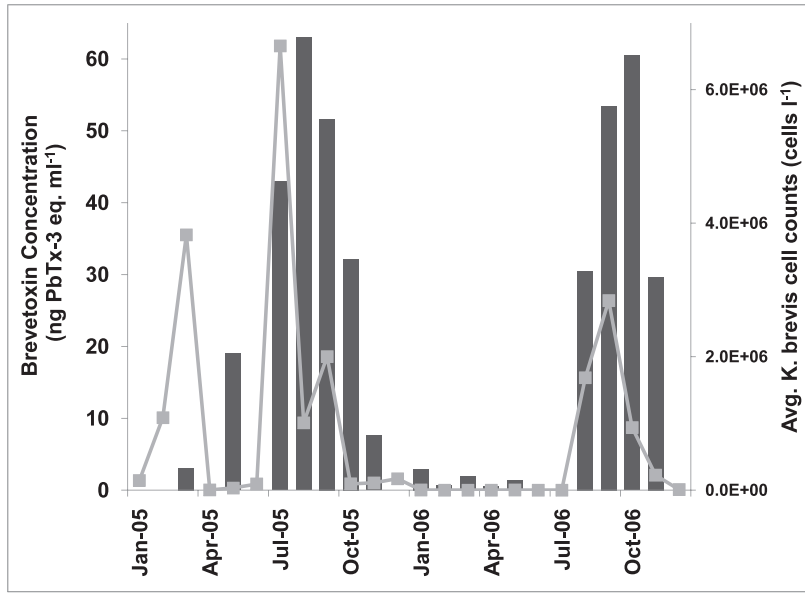

Figure 3. Average brevetoxin concentrations in blood (ng brevetoxin-3 eq/ml) from live stranded sea turtles by month ($n = 50$). Dark grey bars represent average monthly blood concentrations; grey squares represent average monthly *Karenia brevis* cell counts (cells/L) at New Pass, Sarasota Bay.

with elevated blood brevetoxin concentrations stranded predominantly in the summer of 2005 and fall of 2006 (Fig. 3). Many brevetoxin-positive live sea turtles presented floating ($n = 27$). Additionally, several animals were unable to submerge, were entangled, or boat-struck ($n = 9$), were found in the surf ($n = 7$), and/or on the beach ($n = 9$). Among live animals testing positive ($n = 52$) for brevetoxin by ELISA, 29% (2 of 7) of green turtles, 83% (5 of 6) of Kemp's ridley turtles, and 36% (14 of 39) of loggerhead turtles were successfully released. Among live stranded turtles that died or were released there was no significant difference upon admission in brevetoxin concentrations in plasma ($n = 45$, $P = 0.814$, Kruskall-Wallis test) or whole blood ($n = 44$, $P = 0.740$).

Serial plasma samples were taken from 18 live loggerhead sea turtles during rehabilitation and toxin concentration decreased in the blood within 5–80 days postadmission depending upon the initial brevetoxin value (Fig. 4). One green and two Kemp's ridley turtles also had serial blood samples collected and these animals showed faster clearance times of 2–15 days as compared to the loggerheads (data not shown).

Testing of biological fluids and tissues for the presence of brevetoxin by ELISA found toxin present in a least one sample from 42 of 43 (98%) dead stranded sea turtles (Tables 2, 3). Data for urine and small and large intestinal fluids are not shown. In addition, 29 turtles died during rehabilitation and all animals had detectable concentrations of brevetoxin in at least one biological fluid and/or tissue (Tables 2, 3).

In tissues from dead stranded animals and live stranded animals that died, there were significant differences between brevetoxin concentrations in feces ($n = 54$, $P = 0.011$, Kruskall-Wallis test), kidney ($n = 69$, $P = 0.0001$), liver ($n = 69$, $P = 0.0001$), lung ($n = 53$, $P = 0.0001$), and stomach contents ($n = 57$, $P = 0.0004$) with higher mean brevetoxin concentrations in dead stranded animals. However, there was no difference in brevetoxin concentrations in bile, large intestinal fluid, small intestinal fluid, and urine. Among dead stranded animals there were significant differences between loggerhead and Kemp's ridley turtles, with Kemp's having significantly higher brevetoxin concentrations in feces ($n = 29$, $P = 0.027$, Kruskall-Wallis test), kidney ($n = 35$, $P = 0.0001$), liver ($n = 36$, $P = 0.003$), and lung ($n = 33$, $P = 0.001$). There were no differences detected among the other species tested. In addition, for individual biological fluids or tissues tested from dead stranded animals, feces, urine, liver, and kidney had the highest frequency of detecting brevetoxin, whereas in animals that died during rehabilitation premortem blood, feces, bile, small intestinal

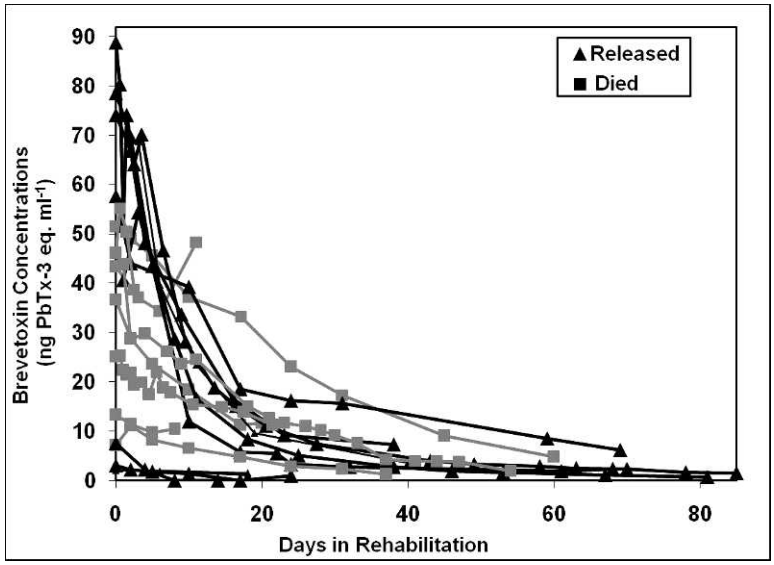

Figure 4. Clearance of brevetoxin from plasma in naturally exposed loggerhead sea turtles ($n = 18$). Sea turtles that died or were euthanized are in grey, animals that were released are marked in black.

fluid, and liver had the highest frequency of detecting brevetoxin (Table 4).

Samples from 12 sea turtles (three premortem and nine postmortem) were analyzed using LC-MS (Table 5). In only one animal (ST 0538) where brevetoxin was measured by ELISA was PbTx-3 not detected by LC-MS in at least one sample tested. The contribution of PbTx-3 to the total toxin measured was variable, ranging from 0% up to 55% of the concentrations measured by ELISA. In addition, seven turtles had detectable levels of the brevetoxin metabolites PbTx-A cysteine conjugate, and five had detectable levels of the cysteine sulfoxide conjugate. Seven turtles also had detectable levels of the PbTx-B cysteine conjugate and cysteine sulfoxide conjugate.

Postmortem examinations were performed on 73 sea turtles and of these, 37 had histologic samples collected and analyzed. The most prevalent gross postmortem finding was moderate to severe decomposition ($n = 35$) and malnutrition ($n = 19$), with malnutrition predominantly found in live stranded animals ($n = 17$). No specific histologic lesions attributable to brevetoxin were noted and the three most common histologic findings were multicentric granulomas with intralesional trematode ova (spirorchid infection; $n = 34$), hepatic atrophy or serous atrophy of fat ($n = 16$), and pneumonia ($n = 15$). In dead stranded turtles with gastrointestinal contents present, these contents consisted of predominantly crabs in loggerhead ($n = 17$) and Kemp's turtles ($n = 8$), although many samples were mixed and included clam, fish, mussel, snail, sponge, and sea star remains. Green turtles' ($n = 9$) gastrointestinal contents consisted of plant material and in a few cases also contained some fish remains. All fish remains consisted mostly of hard parts and no fish tissues were tested for brevetoxin concentrations.

Acute brevetoxicosis was considered the probable cause of death in live sea turtles that presented debilitated during *K. brevis* bloom periods with clinical neurologic signs, and had detectable premortem brevetoxin levels in two or more samples and no other significant pathologic findings. In dead sea turtles, acute brevetoxicosis was considered the cause of death for turtles with at least two positive samples, $\geq$50% of all samples testing positive for brevetoxin, and no other significant pathologic findings. Primary cause of death was attributed to brevetoxicosis in 78% (57 of 73) of sea turtles necropsied. Additionally, six animals with acute brevetoxicosis had evidence of premortem human interaction including two animals with crab trap entanglements and four with boat strikes.

DISCUSSION

Overall 95% (94 of 99) of sea turtles tested were positive for brevetoxin in at least one fluid or tissue. All Kemp's ridley turtles were positive (26 of 26) for brevetoxin, whereas 95% (56 of 59) of loggerheads and 86% (12 of 14) of green turtles were positive. The two ELISA methods were

Table 2. Brevetoxin concentrations (ng brevetoxin-3 eq/g) in tissues at necropsy from dead stranded ($n = 43$) and live stranded sea turtles that died in rehabilitation ($n = 29$) during red tide conditions in 2005–2006 ($n = 72$).[a]

Species	Days in rehab	Lung				Liver				Kidney			
		+/N	Mean	Median	Range	+/N	Mean	Median	Range	+/N	Mean	Median	Range
Dead stranded													
Green	0	3/5	144	106	<ld–396	3/6	186	239	<ld–345	4/6	90	82	<ld–200
Kemp's ridley	0	19/19	131	123	<ld–247	20/20	297	195	<ld–1,006	20/20	151	139	<ld–348
Loggerhead	0	12/15	63	21	<ld–438	17/17	131	71	<ld–683	14/16	63	35	<ld–408
Live stranded													
Green	1–96	0/2	<ld			0/5	<ld		<ld	0/5	<ld		
Kemp's ridley	64	0/1	<ld			0/1	<ld			0/1	<ld		
Loggerhead	1–64	6/13	11	2.5	<ld—32	20/22	43	21	<ld–470	11/23	17	2.5	<ld–138

[a] +/N = number of positive animals out of total animals tested. <ld = below level of detection, some turtles had only one tissue tested.

Table 3. Brevetoxin concentrations (ng brevetoxin-3 eq/g) in biological fluids at necropsy from dead stranded ($n = 40$) and live stranded sea turtles that died in rehabilitation ($n = 24$) during red tide conditions in 2005–2006 ($n = 64$).[a]

Species	Days in rehab	Stomach contents				Feces				Bile			
		+/N	Mean	Median	Range	+/N	Mean	Median	Range	+/N	Mean	Median	Range
Dead stranded													
Green	0	4/6	677	765	<ld–1,312	3/3	968	935	<ld–1,126	1/1	220		
Kemp's ridley	0	19/19	578	578	<ld–3,522	17/17	545	285	<ld–1,832	NT			
Loggerhead	0	9/15	142	27	<ld–971	13/13	402	145	<ld–3,139	2/3	23	35	<ld–50
Live stranded													
Green	1–96	0/5	<ld	<ld	<ld	4/5	102	47	<ld–382	3/4	14	17	<ld–23
Kemp's ridley	64	NT				1/1	22.8			NT			
Loggerhead	1–64	7/15	865	2.5	<ld–11,804	15/17	467	43	<ld–2,012	15/20	50	23	<ld–427

[a] +/N = number of positive animals out of total animals tested. <ld = below level of detection. NT = sample not taken, some turtles had only one fluid tested.

Table 4. Percentage of necropsied sea turtles positive for brevetoxin by sample type during a red tide in 2005–2006 ($n = 72$).[a]

Tissue or fluid	Positive (%)	*N*
Dead stranded		
Urine	100	4
Feces	100	33
Liver	95	43
Kidney	90	42
Lung	87	39
Stomach contents	85	40
Small intestinal fluid	80	5
Bile	75	4
Large intestinal fluid	50	4
Live stranded (died in rehab)		
Urine	70	10
Feces	87	23
Liver	71	28
Kidney	38	29
Lung	38	16
Stomach contents	37	19
Small intestinal fluid	75	16
Bile	75	24
Large intestinal fluid	60	10
Premortem blood	87	23

[a] *N* = total number of sea turtles tested.

successful in detecting brevetoxin immunoreactivity in biologic fluids and tissues from 93% of live and 98% of dead stranded turtles tested. The mean brevetoxin concentrations found in sea turtle biologic fluids and tissues were similar to those found in stranded dolphins, higher than those found in live birds, and lower than those found in prey finfish and stranded sharks.[5,6,7,10]

Live sea turtles with clinical neurologic signs of brevetoxin intoxication were positive for brevetoxin in blood, urine, feces, or some combination of these. The clearance of this toxin from the blood was much slower in sea turtles than that reported previously in rats and mice.[3,34] However, more recent studies have shown that rats treated with PbTx-2 retained higher toxin levels in whole blood than those treated with PbTx-3, which is attributable in part to formation of longer-lasting metabolites.[27] In the naturally exposed sea turtles, brevetoxin was eliminated to low levels (<10 ng/g) after 5 to 60 days in loggerhead turtles and 2 to 15 days in green and Kemp's ridley turtles. This suggests that a natural exposure of sea turtles to brevetoxins may be associated with a longer time for elimination. Indeed, a recent study on striped

Table 5. Brevetoxin concentrations (ng/g) and composition of samples analyzed by liquid chromatography with mass spectrometry ($n = 12$).[a]

ID number	Species	Tissue type	PbTx-3	Cysteine PbTx-A	Cysteine PbTx-A sulfoxide	Cysteine PbTx-B	Cysteine PbTx-B sulfoxide	ELISA value (ng PbTx-3 eq/g)
ST0541	Green	Postmortem feces	100	46	54	419	24	1,126
ST0541	Green	Stomach contents	104	14	76	107	<ld	464
ST0569	Green	Postmortem feces	<ld	<ld	<ld	317	<ld	935
ST0569	Green	Stomach contents	198	27	68	271	45	1,216
ST05118	Green	Postmortem feces	<ld	<ld	<ld	<ld	<ld	841
ST05118	Green	Stomach contents	440	<ld	<ld	<ld	<ld	1,066
ST0538	Kemp's ridley	Liver	<ld	<ld	<ld	<ld	<ld	143
ST0542	Kemp's ridley	Liver	60	<ld	<ld	<ld	<ld	398
ST0542	Kemp's ridley	Stomach contents	140	<ld	<ld	<ld	<ld	593
ST0542	Kemp's ridley	Muscle from fish	<ld	<ld	<ld	<ld	<ld	670
ST0567	Kemp's ridley	Postmortem feces	<ld	115	157	181	62	1,046
ST0567	Kemp's ridley	Liver	<ld	<ld	<ld	102	<ld	564
ST0567	Kemp's ridley	Stomach contents	452	161	69	509	89	1,559
ST05159	Kemp's ridley	Stomach contents	2,776	<ld	<ld	<ld	<ld	5,146
ST05159	Kemp's ridley	Small intestinal fluid	473	<ld	<ld	<ld	<ld	1,717
ST05151	Loggerhead	Stomach contents	100	<ld	<ld	<ld	<ld	308
ST0694	Loggerhead	Premortem feces	424	5,135	256	10,521	539	61,078
ST06132	Loggerhead	Premortem feces	248	44	<ld	959	76	2,959
ST06134	Loggerhead	Premortem feces	285	137	<ld	2,118	240	2,483
ST06138	Loggerhead	Liver	258	<ld	<ld	47	<ld	469
ST06138	Loggerhead	Stomach contents	155	63	56	303	30	11,804
ST06138	Loggerhead	Small intestinal fluid	347	<ld	<ld	625	<ld	2,117
ST06138	Loggerhead	Large intestinal fluid	<ld	<ld	<ld	98	<ld	356
ST06138	Loggerhead	Bile	<ld	<ld	<ld	<ld	<ld	427

[a] PbTx = brevetoxin. <ld = below level of detection. ELISA = enzyme-linked immunosorbent assay.

mullet (*Mugil cephalus*) exposed to aqueous *K. brevis* cells likewise determined that blood levels of toxin was reduced to low levels after 12.5 days.[33] In addition, the majority of sea turtles that survived rehabilitation had eliminated 80% of the toxin from their blood by day 20, whereas animals that died during rehabilitation had eliminated only 40–60% of the toxin by day 20.

The slower metabolism of reptiles may also contribute to the slower brevetoxin elimination as compared to mammals.[25] However, it was interesting that Kemp's ridley and green turtles appeared to clear the toxin faster than loggerhead turtles. The loggerheads in this study were larger than the Kemp's ridley and green turtles. One possible reason for this species difference in clearance of toxin may be due to differences in body mass and subsequent differences in metabolism and distribution of the toxin between species. Although these preliminary data suggest there may be species differences in the clearance of brevetoxin between loggerheads and other sea turtle species, the sample sizes for Kemp's ridley and green turtles were small.

The three turtle species also differ in diet preference and could have had different initial toxin loads depending upon prey species or forage type consumed. As adults, loggerhead and Kemp's ridley sea turtles are predominantly omnivores and consume fish and invertebrate species whereas green sea turtles are predominantly herbivores feeding on sea grass; however, juvenile animals may have different foraging habits. All of the Kemp's ridley and green turtles examined in this study were subadults. Recent research has found that fish, benthic invertebrates, and sea grass can all bioaccumulate significant concentrations of brevetoxin and serve as vectors to aquatic animals.[4,7,19,24] Therefore more research is needed to determine how individual species of turtles, including different age classes, are exposed to brevetoxin via their prey and how this may affect their risk of intoxication.

Another interesting finding regarding the clearance of the toxin was the continued presence of brevetoxin in tissues and body fluids from animals that died during rehabilitation. Animals that died during rehabilitation had lower brevetoxin tissue burdens in lung, kidney, and liver than dead stranded turtles. In experimental studies of rats, brevetoxin was found to be metabolized by the liver and kidney after ingestion and excreted in the feces and urine.[3] Turtles undergoing rehabilitation followed this pattern by continuing to excrete the toxin through the gastrointestinal tract resulting in higher brevetoxin levels in bile, small and large intestinal fluids, and feces than in liver and kidney in animals that died. Studies with rat liver cells indicate that brevetoxin is metabolized by oxidation, reduction, epoxidation, and conjugation[26] and that the products of these reactions still retain substantial biological potency.[2,28] Hence, it is likely that in some animals that died toxin was remetabolized from the intestinal tract to the circulation leading to continued intoxication while these animals were undergoing rehabilitation.

For animals that were examined histologically there were no definitive lesions associated with brevetoxin exposure. Unlike some harmful algal toxins, such as domoic acid,[29] brevetoxins may not cause specific pathologic changes in the brain. However, systematic documentation of the pathologic changes associated with brevetoxin intoxication in wildlife is currently lacking.[20] This deficiency cannot be addressed without performing an experimental investigation with controlled dosing of brevetoxin, which is not possible with threatened and endangered sea turtles.

Although brevetoxin-specific pathologic lesions were not found, several consistent lesions were considered related or secondary. Malnutrition noted in many turtles was presumably related to impaired or absent foraging activity subsequent to weakness or comatose condition typically noted in intoxicated animals. Similarly, brevetoxin-induced weakness or paralysis may have been the cause of aspiration pneumonia noted in some cases. Some turtles in rehabilitation were noted to be unable to raise their heads normally to breathe (Manire, unpubl. data) and aspiration of seawater seemed a probable outcome in some live and dead stranded individuals.

In conclusion, brevetoxin exposure was documented in 95% (94 of 99) of dead and live sea turtles stranding during the 2005 and 2006 *K. brevis* bloom events off the west coast of Florida. Dead stranded sea turtles had significantly higher brevetoxin concentrations in tissues than live stranded animals. Live stranded sea turtles with neurologic signs had significant concentrations of brevetoxin in their fluids and tissues and in the majority of these animals brevetoxin exposure was determined to have caused or contributed to the strandings.

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