

## PROGRAMMATIC REPORT FORMAT

**Grantee:** Mote Marine Laboratory

**Contract Number:** Sea Turtle Grant #05-016-R

**Project Title:** Investigation of brevetoxin induced morbidity and mortality in stranded sea turtles from central west Florida

**Report Period:** May 1, 2005-April 30, 2006

**Project Manager(s) Principle Investigator(s):**

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**Tasks:** Objective 1 - To determine brevetoxin levels in tissues and body fluids from stranded sea turtles during red tide events and in the absence of red tide.

Objective 2 - To determine the spectrum of gross and histologic lesions that may be associated with the presence of brevetoxin in tissues from stranded sea turtles.

Objective 3 - To investigate if brevetoxin levels in stranded animals are associated with *K. brevis* cell counts and investigate any associations with the covariates of season, stranding location, gender, reproductive condition, and body condition.

**Accomplishments:**

**Objective 1-**

From January 1 to December 31, 2005, sea turtle strandings in Mote Marine Laboratory's (MML) stranding area increased 4-fold (average 44 animals year) with 174 turtles stranding during the period. During this period 141 animals were recovered dead, and 33 animals were recovered live with two live animals released at the stranding site and not brought into rehabilitation. There were 97 loggerhead turtles (*Caretta caretta*), 50 Kemp's ridley turtles (*Lepidochelys kempi*), 19 green turtles (*Chelonia mydas*), 3 hawksbill turtles (*Eretmochelys imbricata*) and three turtles of undetermined species recovered.

Live turtles admitted for rehabilitation (n=31) had swollen eyes and neurologic clinical signs including unresponsiveness, inability to move the flippers, and circling. Testing of blood and body fluids for the presence of brevetoxin by ELISA found toxin present in 17 of 22 (77%) live sea turtles (Table 1). Brevetoxin levels at admission ranged from 4.1 to 88.8 ng/ml in plasma (n=14), 3.1 to 82.0 ng/ml in whole blood (n=13) and 29.5 to 728.7 ng/g in feces (n=5). Serial plasma samples were taken from five live loggerhead sea turtles during rehabilitation and toxin was cleared from the blood within 5-40 days post-admission depending upon the initial brevetoxin value. One green and one Kemp's ridley turtle also had serial blood samples collected and these animals showed faster clearance times of 2-15 days as compared to the loggerheads.

Testing of blood and tissues for the presence of brevetoxin by ELISA found toxin present in 12 of 13 (92%) dead sea turtles (Table 2). In addition, of the 31 live animals recovered, 15 died during rehabilitation and all (15/15, 100%) had detectable levels of brevetoxin in blood and

tissue (Table 2). Mean values for brevetoxin levels by tissue type for dead stranded and animals that died in rehabilitation are presented in Table 3. Overall, animals that died in rehabilitation had lower mean brevetoxin values than dead stranded animals in most tissues tested. For individual tissue types tested, feces and urine were the most sensitive samples for detecting brevetoxin, followed by large intestine fluid, small intestine fluid, liver and bile (Table 4).

### Objective 2 –

Post-mortem examinations were performed on 57 sea turtles. Of these animals, 28 animals had tissues tested for brevetoxin, and toxin was present in 27 of 28 turtles (96%). When evaluating sea turtles the detection limit for the ELISA is 5 ng/g for tissues and <2 ng/ml for plasma. Animals with values above these concentrations were considered exposed. Brevetoxin intoxication was considered the probable cause of death in animals that presented live with detectable brevetoxin levels or in dead animals with three or more tissues above the detection limit and no other significant pathologic findings. For all positive animals (n=27) brevetoxin intoxication was determined to be the primary cause of death.

Of the 28 animals tested for brevetoxin, 21 turtles had histologic samples collected and analyzed. Preliminary evaluations of histopathology from these animals found no specific histologic lesions attributable to brevetoxin. The one brevetoxin negative animal was an emaciated green turtle with severe fibropapilloma tumors. Of the remaining 20 brevetoxin positive animals, seven were considered severely emaciated at death, four had secondary human interactions involving boat strikes or entanglements, three had secondary infections or ruptures of the gastrointestinal system, and one had secondary aspiration pneumonia.

### Objective 3 –

The proportion of animals positive for red-tide exposure was consistent among species, gender and age class, but sample sizes were small and this should be re-examined when additional samples are available. Statistical tests ( $\chi^2$  for RxC contingency table) did not show significant differences among species (p=0.20), gender (p=0.10) or age class (p=0.10). However the proportion of animals positive for red-tide exposure (Figure 5) was different among seasons (p=0.025). This seasonal difference correlated to the increase in red-tide positive animals during the months of July, August and September at the peak of the sea turtle strandings (Figure 1). Differences between brevetoxin levels in tissues from live animals that died and dead stranded animals were analyzed using the Kruskal-Wallis test. There were significant differences between brevetoxin levels in lung (p=0.001), liver (p=0.002), kidney (p=0.001), and stomach contents (p=0.040) between dead stranded turtles and turtles that died in rehabilitation, however, there was no difference in feces (p=0.380).

The bloom index ranged from a low of 0 to a high of 51,513,533 *K. brevis* cells/L. The KWANOVA conducted on brevetoxin positive turtles compared to brevetoxin negative turtles found no difference in the bloom index ( $H_{0: \mu_1 = \mu_2} = 2.41, p=0.12$ ). However, when the test was conducted for live stranded (median=81,824) vs. dead stranded turtles (median=594,001) there was a significant difference ( $H_{0: \mu_1 = \mu_2} = 5.63, p=0.0176$ ), with dead stranded animals having a greater than 6-fold higher bloom index than live stranded animals.

In conclusion, brevetoxicosis appeared to be the primary cause of stranding in 34 of 40 (85%) live or dead stranded animals during 2005 off the west coast of Florida during a severe red

tide event. Although additional animals were not tested it can be assumed that a similar proportion of animals stranded during this time were also affected by brevetoxin intoxication. Animals stranded dead during periods of high red tide cell counts while live animals stranded during periods with lower cell counts indicating that there is a threshold at which red tide exposure becomes fatal. Findings from this study are only preliminary, but the fact that the majority of the live and dead animals sampled during the 2005 red tide event were positive for brevetoxin indicates that brevetoxin intoxication may play a larger role in the morbidity and mortality of sea turtles off the west coast of Florida than previously recognized.

**Presentations:**

D. Fauquier, L. Flewelling, C. Manire, J. Gannon, V. Socha, A. Foley, J. Landsberg. 2006. Preliminary investigation of brevetoxin induced morbidity and mortality in stranded sea turtles from central west Florida. 2006 Marine Turtle Permit Holder Workshop, Jacksonville, Florida.

D. Fauquier, L. Flewelling, C. Manire, J. Ramsdell, J. Gannon, M. Kinsel, B. Stacy, N. Barros, V. Socha, J. Grimes, A. Foley, R. Pierce, J. Landsberg. 2006. Brevetoxin induced morbidity and mortality in stranded bottlenose dolphins and sea turtles from central west Florida in 2005. The Southeast and Mid-Atlantic Marine Mammal Symposium, Ft. Lauderdale, Florida.

C.A. Manire, D. Fauquier, J. Cianciolo, L. Flewelling, B. Stacy, A. Foley, and J. Landsberg. 2006. Red tide toxicosis in sea turtles. 37<sup>th</sup> Annual International Association of Aquatic Animal Medicine Conference, Nassau, Bahamas.

**Additional Funding**

Additional funding was received from the Sea Turtle Grant Program for the second year of this study. A proposal to continue this work and to begin work with stranded sea birds was submitted to Morris Animal Foundation in April 2006.

**Table 1: Pre-mortem brevetoxin levels in fluids and tissues from live sea turtles analyzed by ELISA (n=22).**

[All values in ng/g (blood=ng/ml); NEG=None detected (LD<2 ng/ml for plasma, LD<0.4 ng/ml for whole blood); LD=Detection limit. CC=Loggerhead, CM=Green, LK=Kemp's ridley]

ST ID	STR DATE	Species	Plasma	Whole Blood	Feces	Outcome
ST 0513	3/30/2005	LK	*	3.1	*	Died
ST 0516	4/4/2005	LK	*	NEG	*	Released
ST 0517	4/14/2005	CC	*	NEG	*	Released
ST 0522	5/16/2005	CC	*	19.0	*	Died
ST 0530	6/4/2005	CC	*	NEG	*	Released
ST 0532	6/15/2005	CC	*	NEG	*	Released
ST 0549	7/3/2005	CC	*	43.0	*	Died
ST 0568	8/1/2005	LK	78.4	35.0	389.9	Released
ST 0574	8/6/2005	LK	*	82.0	728.7	Released
ST 0576	8/7/2005	CC	75.3	50.5	*	Died
ST 0587	8/10/2005	CC	64.2	70.0	*	Died
ST 0590	8/10/2005	CC	23.5	13.0	*	Died
ST 05100	8/13/2005	CC	54.6	28.0	*	Died
ST 05141	9/15/2005	CC	25.2	*	29.5	Died
ST 05142	9/16/2005	CC	18.5	*	31.5	Released
ST 05144	9/17/2005	LK	74.0	*	722.0	Rehab
ST 05146	9/27/2005	CC	88.8	*	*	Released
ST 05151	10/9/2005	CC	46.2	*	*	Died
ST 05153	10/9/2005	CM	4.1	*	*	Released
ST 05158	10/12/2005	CC	46.2	*	*	Died
ST 05170	11/28/2005	CC	7.6	*	*	Rehab
ST 05174	12/27/2005	CM	NEG	*	*	Released

**Table 2: Brevetoxin levels in fluids and tissues from dead stranded turtles and turtles that died during rehabilitation analyzed by ELISA (n=28).**

[All values in ng/g (fluids=ng/ml): NEG=None detected (LD<5 ng/g for tissues, LD< 2 ng/ml for bile); LD=Detection limit, CC=Loggerhead, CM=Green, LK=Kemp's ridley, L=Live Strand Died in Rehab, D=Dead Strand, S: Cnts=Stomach Contents, LI Fluid=large intestine fluid; SI Fluid=small intestine fluid]

ST ID	Str Date	Rec Date	Days in Rehab	Species	Live	Dead	Lung	Liver	Kidney	St Cnts	Bile	SI fluid	LI fluid	Feces
ST 0501	1/11/2005	1/11/2005	0	CM	D	NEG	NEG	NEG	NEG					
ST 0505	2/28/2005	3/1/2005	0	LK	D	108.7	101.2	114.3	175.3					
ST 0525	5/21/2005	5/22/2005	0	CC	D	14.8	27.0	NEG	16.8					21.9
ST 0538	6/18/2005	6/19/2005		LK	D	63.7	143.1	61.1	113.1			154.1		159.8
ST 0542	6/21/2005	6/22/2005	0	LK	D	89.0	307.2	145.0	893.0					641.7
ST 0566	7/31/2005	7/31/2005	0	LK	D	174.0	293.0	142.3	782.0					231.5
ST 0567	8/1/2005	8/2/2005	0	LK	D	185.0	564.1	348.8	1559.2					1045.0
ST 0575	8/6/2005	8/7/2005	0	LK	D	219.2	392.7	298.8						
ST 0580	8/8/2005	8/9/2005	0	LK	D	208.5	291.2	293.0	1036.5					1832.2
ST 0583	8/9/2005	8/9/2005	0	CC	D	26.5	19.5	15.3	NEG					20.0
ST 0593	8/11/2005	8/11/2005	0	CC	D	21.0	27.3	35.0	NEG					268.4
ST 0595	8/11/2005	8/11/2005	0	CC	D	NEG	53.3	33.5	26.7					33.0
ST 05103	8/13/2005	8/14/2005	0	LK	D	124.8	358.6	158.1	490.1					285.0
ST 0513	3/30/2005	6/2/2005	65	LK	L	NEG	NEG	NEG						22.8
ST 0522	5/16/2005	5/25/2005	10	CC	L	NEG	8.0	NEG						72.0
ST 0524	5/17/2005	5/19/2005	3	CM	L	NEG	NEG	NEG	NEG					70.0
ST 0549	7/3/2005	7/7/2005	5	CC	L	NEG	33.4	NEG	NEG					43.3
ST 0576	8/7/2005	8/9/2005	3	CC	L	31.0	74.8	79.2	224.1					
ST 0587	8/10/2005	8/14/2005	5	CC	L	29.6	28.5	25.7	10.7	140.4				1007.0
ST 0590	8/10/2005	8/19/2005	10	CC	L	NEG	13.5	NEG		30.4				13.0
ST 05100	8/13/2005	8/17/2005	5	CC	L	NEG		10.0	24.2	10.0	23.0	10.0		184.0
ST 05116	8/18/2005	8/19/2005	2	CC	L	NEG	17.2	NEG	NEG	4.5				3.0
ST 05123	8/20/2005	8/30/2005	11	CC	L	NEG	NEG	NEG		NEG	NEG	NEG		13.5
ST 05124	8/20/2005	8/24/2005	5	CC	L	21.0	25.3	20.4	NEG	33.9	20.2	417.5	2012.1	
ST 05125	8/21/2005	8/23/2005	3	CC	L	19.4	26.0	NEG	NEG	5.9	21.7	1178.5	504.2	
ST 05141	9/15/2005	10/7/2005	23	CC	L	14.0	27.7	12.5	NEG	46.8	8.1	13.8	74.5	
ST 05151	10/9/2005	10/13/2005	5	CC	L	6.1	16.4	13.0	308.0	NEG	24.9	474.7	1678.1	
ST 05158	10/12/2005	12/8/2005	59	CC	L	NEG	NEG	NEG		23.0	8.4	29.2	28.0	

**Table 3: Mean brevetoxin levels for each tissue type tested from dead stranded turtles and turtles that died during rehabilitation.**

[All values in ng/g (fluids=ng/ml). St Cnts=Stomach Contents. LI Fluid=large intestine fluid; SI Fluid=small intestine fluid]

Dead Stranded	Lung	Liver	Kidney	St Cnts	Feces	Bile	SI fluid	LI fluid
Mean	98.21	205.72	122.75	401.44	454.47			
Stdev	79.79	185.98	114.84	507.16	581.22			
N	13	13	13	12	10	0	0	1
Dead Rehab	Lung	Liver	Kidney	St Cnts	Feces	Bile	SI fluid	LI fluid
Mean	11.39	20.88	13.58	59.70	435.49	30.28	15.19	303.67
Stdev	9.66	18.55	18.50	110.70	717.49	41.58	9.35	436.68
N	15	14	15	10	14	10	7	7

**Table 4: Percent of dead animals positive for brevetoxin by tissue type**

[LI Fluid=large intestine fluid; SI Fluid=small intestine fluid]

Tissues Fluids	% Positive	N
Feces	100%	24
Urine	100%	4
LI Fluid	88%	8
SI Fluid	86%	7
Liver	82%	27
Bile	80%	10
Muscle	75%	4
Kidney	61%	28
Lung	61%	28
Stomach Contents	59%	22
Brain	50%	2