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Introduction

All species of sea turtles in United States waters are listed on the Endangered Species Act of 1973 as endangered or threatened. Scientific research on these species has primarily concentrated on threats to nesting beaches, interactions with commercial fisheries, and the effect of pollutants on sea turtle populations (Mckenzie *et al.*, 1999, Rumbold *et al.*, 2001, Tomas *et al.*, 2002, Keller *et al.*, 2003). Little is known about disease and other natural factors that affect sea turtle populations. As long-lived animals, sea turtles can be indicator species alerting scientists to new and emerging diseases affecting wild populations. An understanding of risks to these populations including the effects of disease and other natural factors is vital to the recovery of these species (Cleaveland *et al.*, 2002).

Harmful algal blooms are suggested to have caused or contributed to mortality events in marine mammals, sea turtles and sea birds documented over the last twenty years in the world's oceans (Geraci, 1989, Work *et al.*, 1993, Sierra Beltran *et al.*, 1997, Foote *et al.*, 1998, Scholin *et al.*, 2000). In Florida waters the most prevalent harmful algae is the dinoflagellate *Karenia brevis,* which produces brevetoxins. Brevetoxins can cause acute respiratory and neurologic symptoms including death through inhalation and/or ingestion (Steidinger *et al*., 1973). The possible chronic effects of repeated brevetoxin exposure are unknown but may include impaired immune function, reduced growth and reproduction, morbidity, or mortality (Landsberg, 2002).

High mortality due to brevetoxicosis from *K. brevis* has been documented in fish and manatees along the western coast of Florida (Gunter *et al.*, 1948, Bossart *et al.*, 1998, Landsberg and Steidinger, 1998, Landsberg, 2002). In 2002, 2003, and 2005 over 170 manatees died from red tide intoxication (Flewelling *et al.,* 2005, FWC unpublished data). Significant increases in mortality of dolphins, sea birds and sea turtles have been documented during red tide events with recent evidence implicating brevetoxin involvement (Gunter *et al.*, 1948, Geraci, 1989, Foote *et al.*, 1998, Kreuder *et al.*, 2002; Van Dolah *et al.,* 2003, Flewelling *et al.*, 2005). A competitive enzyme-linked immunosorbent assay (ELISA) has recently been developed by Naar *et al.* (2002), which quantifies the amount of brevetoxin present in biologic tissues and fluids. In March 2004, there was an unusual mortality event involving bottlenose dolphins along the coast of the Florida panhandle near St. Joseph Bay. Over 100 animals were involved in the event and preliminary ELISA testing by the Florida Fish and Wildlife Research

Institute found elevated stomach content brevetoxin levels (536.0 - 6176.0 ng/g) in more than 30 animals (NOAA/FWC interim report, 2004; Flewelling *et al.,* 2005).

More recently there were severe and prolonged red tide events off the west coast of Florida from February through December 2005 and from August through December 2006. *Karenia brevis* cell concentrations reached levels of 100 million cells per liter of seawater, indicating strong red tide events. As point of reference, concentrations of 1000 cells per liter or less are considered background levels, and 100,000 cells per liter or more typically cause respiratory irritation in humans and fish kills. During the 2005 and 2006 red tide blooms there were significant increases in sea turtle strandings. At Mote, sea turtle strandings increased 4-fold in 2005 (n=174) and 3-fold in 2006 (n=144) over the previous 12-year average (43 turtles/year) of turtles stranding in the region. The scope of the 2005 red tide event was much greater in magnitude of severity and spatial distribution than many previous events, prompting the National Marine Fisheries Service to declare a multi-species Unusual Mortality Event for southwest Florida including manatees, dolphins, sea turtles and sea birds.

Red tides are frequent events in southwest Florida and the overall impact on wildlife populations has previously been unknown because of lack of appropriate testing methods. The recent creation of ELISA-based tests has facilitated the determination of tissue levels of brevetoxin in dead stranded manatees and dolphins in Florida. These new testing methods can also be applied to sea turtle species to determine if red tides that are a common natural factor in Florida waters, have a major impact on the health of sea turtle populations.

The two years of this study was a collaborative project between Mote Marine Laboratory (MML), Florida Fish and Wildlife Conservation Commission's Fish and Wildlife Research Institute (FWC/FWRI), National Ocean Service (NOS), University of Illinois (UI), and University of Florida (UF) to investigate the extent that brevetoxin intoxication contributes to morbidity and mortality in stranded sea turtles along the central west coast of Florida. Brevetoxin levels in tissues and body fluids from stranded sea turtles during strong red tide events were determined. The spectrum of gross and histologic lesions that might be associated with the presence of brevetoxin in tissues from stranded sea turtles was investigated. Lastly, relationships between brevetoxin levels in sea turtle tissues and *K. brevis* cell counts, stranding location, gender and/or species were evaluated.

Material and Methods

Live and dead animals consisted of stranded sea turtles that were recovered and brought into rehabilitation or necropsied at MML during 2005 and 2006. Animals were recovered chiefly from Sarasota County, with additional animals from Manatee, Charlotte, Collier, Lee and Pinellas Counties. The main species tested were loggerhead (*Caretta caretta*), Kemp's ridley (*Lepidochelys kempi*) and green (*Chelonia mydas*) sea turtles. Blood samples were collected from most live animals on admission to MML's Sea Turtle Hospital for rehabilitation. Serial blood samples (1-3ml) were collected from a select number of live sea turtles at admission and opportunistically thereafter, ranging from every two days to every two weeks, until clinical signs resolved.

Dead animals, or animals that died during rehabilitation, were necropsied according to techniques described by Wyneken (2001). Body condition and gender were determined at necropsy. Tissues were collected for brevetoxin analysis including lung, liver, kidney, stomach contents, feces, and bile. In a few animals gastrointestinal fluids and urine were also collected. Tissues to be tested by ELISA could be moderately to severely decomposed, making it possible to get samples from many dead stranded turtles examined.

Brevetoxin analysis was performed by Leanne Flewelling at FWRI. To prepare samples for analysis, tissues were extracted twice in 80% aqueous methanol (1:5 w/v). The pooled extracts were

partitioned with hexane. The methanol fraction was retained for analysis. Urine and serum/plasma samples were centrifuged to clarify (if necessary) and extracted with C18 solid phase extraction columns (1 g, Supelco Discovery). A competitive ELISA to detect brevetoxins (PbTx) was performed according to Naar *et al.* (2002). Briefly, 96-well microplates were pre-coated with brevetoxin, and any remaining binding sites in the wells were blocked. Samples and controls were added to the wells and allowed to compete with the plate-bound toxin for anti-brevetoxin antibodies. After one hour, the wells were rinsed; removing the samples and all antibodies except those attached to the plate-bound toxin. The antibodies remaining in the plate were visualized using a peroxidase (HRP) conjugated secondary antibody, and the HRP substrate o-phenylenediamine. This assay recognizes all congeners and metabolites of brevetoxin that have a PbTx-2-type backbone. The lower limit of quantification is 5 ng/g (1 ng/ml for blood/urine).

Additionally, whole blood from some live animals was analyzed for brevetoxin using a direct ELISA optimized for blood collection cards by John Ramsdell and staff at NOAA-NOS according to Maucher *et al.* (2007). Briefly, dried blood samples (~100µl) were eluted from blood cards with phosphate-buffered saline (PBS) with 6% methanol and 100% acetonitrile used to precipitate proteins. This extraction method allowed for linear recovery of the common brevetoxin metabolites PbTx-3, cysteine conjugate (*m/z* 1018) and cysteine sulfoxide conjugate (*m/z* 1034). The ELISA is designed as a direct competitive format with a working range (I_{20}-I_{80}) of 90-2100 pg PbTx-3/mL. The brevetoxin antibody is specific to ring one of type 2 brevetoxins and previously characterized by radioimmunoassay (Woofter *et al.* 2003). The detection limit of the assay is 1.0 ng brevetoxin-3 equivalents per milliliter of blood.

Representative samples of all organ systems from fresh carcasses were collected to survey for concurrent diseases and to attempt to establish a cause of death as part of a comprehensive postmortem evaluation. All tissues were fixed in 10% neutral buffered formalin and submitted to Dr. Michael Kinsel of the University of Illinois Zoological Pathology Program or to Dr. Brain Stacy of the University of Florida for examination.

Due to the low sample size, correlations between brevetoxin levels in animals and gender, species and season were analyzed using the χ^2 test. Differences between brevetoxin levels in tissues from live animals that died and dead stranded animals were analyzed using the Kruskal-Wallis test. *Karenia brevis* cell counts were obtained from Mote Marine Laboratory's Red Tide Monitoring Program (Kirkpatrick, unpublished data). Water samples were collected in two locations in Sarasota Bay, one in New Pass and one adjacent to City Island grassflat. Surface water samples were collected from one to five times per week. Samples were collected in scintillation vials and immediately preserved in Utermohl's solution, and stored in dark, room temperature conditions. Enumeration of *K. brevis* abundance was conducted using an inverted Olympus CK40 microscope following standard protocols (Sellner et al 2003).

Monthly mapping of turtle strandings were created for both 2005 and 2006 to look at patterns of stranding with the bloom. In addition, maps were created with standard deviation ellipses to provide a coarse measure of dispersion of stranding locations for each year. Standard deviation ellipses represent dispersion in two directions and determine the dominant axis of dispersion (Lefever 1926, Gong 2002).

A stepwise multivariate linear regression model was applied to determine the relationship between the number of strandings per week and cell count averages (at New Pass in Sarasota County) for that week; the week prior; the month, two months, and six month time frames (N = 104). The stepwise linear regression finds the best fitting explanatory variable in predicting the value of the dependent variable, then tests for the next best fit, until all variables have been tested. Variables were

included in the model at α=0.05, otherwise were excluded. Where necessary, transformations of the dependent variable were applied to meet regression assumptions.

For all statistical tests, results were considered significant at $p \leq 0.05$. All statistical calculations and GIS mapping were performed using Statistica (StatSoft Inc., Tulsa, Oklahoma, USA), SPSS 11.0 (SPSS Inc., Chicago, Illinois, USA) and/or ArcInfo 9.0 (ESRI, Redlands, California, USA) software.

Results

From January 1, 2005 to December 31, 2006, sea turtle strandings in MML's stranding area increased 3 to 4-fold (average 43 animals/year) with 318 turtles stranding during the period (Figures 1 & 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 seven turtles of undetermined species recovered.

During the height of the red tide blooms some live turtles admitted for rehabilitation 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 40 of 49 (82%) live sea turtles (Table 1). Brevetoxin levels at admission ranged from 0.7 to 106.6 ng/ml in plasma (n=40), 2.4 to 82.0 ng/ml in whole blood (n=28) and 29.5 to 728.7 ng/g in feces (n=5). When evaluating sea turtles the detection limit for the ELISA is <1 ng/ml for plasma/whole blood and 5 ng/g for tissues. Animals with values above these concentrations were considered exposed.

Serial plasma samples were taken from 19 live loggerhead sea turtles during rehabilitation and toxin concentration was decreased in the blood within 5-80 days post-admission depending upon the initial brevetoxin value (Figure 3). Two green and two 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 (Figure 4).

For live animals testing positive for brevetoxin 67% of green turtles (2 of 3), 80% of Kemp's ridley turtles (4 of 5) and 41% of loggerhead turtles (13 of 32) were successfully released or are still undergoing rehabilitation. The percentage of initial brevetoxin levels eliminated from the blood over time in 19 turtles with serial blood samples is shown in Figure 5, which illustrates that animals that survived had quicker elimination of the toxin from blood than animals that died.

Testing of blood and tissues for the presence of brevetoxin by ELISA found toxin present in 16 of 17 (94%) dead sea turtles (Table 2). In addition, of the 61 live animals admitted to rehabilitation, 29 died during rehabilitation and all (29/29, 100%) had detectable levels of brevetoxin in blood and/or tissue (Table 2). Mean values for brevetoxin levels by tissue type for dead stranded animals and animals that died in rehabilitation are presented in Table 3. Overall dead stranded animals had higher mean brevetoxin levels in lung, liver and kidney and lower mean brevetoxin levels in small and large intestinal fluids than animals that died during rehabilitation. In addition, for individual tissues tested from dead stranded animals, feces, bile and liver were the most sensitive samples for detecting brevetoxin, while in animals that died during rehabilitation feces, urine, bile and small intestinal fluid were the most sensitive samples (Table 4).

Post-mortem examinations were performed on 85 sea turtles. Of these animals, 46 animals had tissues tested for brevetoxin, and toxin was present in 45 of 46 turtles (98%). Brevetoxin intoxication was considered the probable cause of death in animals that presented live with detectable brevetoxin levels in blood, or in dead animals with one tissue > 50ng/g brevetoxin or with three or more tissues

above the detection limit of 5ng/g brevetoxin, and no other significant pathologic findings. Of the 46 animals tested for brevetoxin, 37 turtles had histologic samples collected and analyzed. Preliminary evaluations of histopathology from these animals found no specific histologic lesions attributable to brevetoxin; however 51% of the animals examined had evidence of pneumonia and/or emaciation on histopathology. Cause of death was attributed to primary red tide intoxication in 41 of 45 turtles (91%).

The proportion of animals positive for red-tide exposure (Figure 6) was consistent among species and gender, but sample sizes were small for green and Kemp's ridley turtles and this should be re-examined when additional samples are available. Statistical tests (χ^2 for R×C contingency table) did not show significant differences among species (p=0.114) and gender (p=0.073). However the proportion of animals positive for red-tide exposure was different among seasons (p=0.014). 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. 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=0.002), liver (p=0.002), and kidney (p=0.0001) between dead stranded turtles and turtles that died in rehabilitation, however, there was no difference in stomach contents, feces, bile, small intestinal fluid, large intestinal fluid, and urine.

The study area experienced a bloom of red tide from February through December 2005 and from August through December 2006 (Figure 7), with high counts (>100,000 cells/liter) occurring in 2005 from mid-Jan to mid-Mar and again from late May-mid October. In 2006, high counts occurred from August through November. Monthly mapping of turtle strandings also followed this pattern of the bloom (Figures 8 & 9). Standard deviation ellipses suggest strandings were further north in 2005 than in 2006; and were less dispersed in 2005 than in 2006 (Figure 10).

A square root transformation was applied to meet the regression assumptions. Only two variables, average weekly cell counts and average monthly cell counts, were found significant in predicting the number of strandings. All other variables were excluded in the stepwise algorithm. Overall model utility was significant (F=8.557, p = 000), although the adjusted R^2 (0.128) suggested weak correlation (Table 5). A Pearson Correlation was applied to test for collinearity between the weekly average counts and monthly average counts; R^2=0.192, p=0.51. This, not surprisingly, suggests some collinearity between the two variables.

Discussion

Both the ELISA methods were successful in detecting brevetoxin in tissues and body fluids from 89% of live (50 of 56) and 94% (16 of 17) of dead stranded turtles tested. Live sea turtles with clinical neurologic signs of brevetoxin intoxication were positive for brevetoxin in blood and/or feces. The clearance of this toxin from the blood was much slower in sea turtles than that reported previously in rats and mice (Cattet and Geraci, 1993, Woofter *et al.*, 2003). 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 (Radwan *et al.*, 2005). In our naturally exposed sea turtles, brevetoxin was eliminated to background levels (<10ng/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 to brevetoxins may be associated with a longer time for elimination. Indeed, a recent study on stripped mullet exposed to aqueous *K. brevis* cells, likewise determined that blood levels of toxin reduced to background levels (2.25 nM) after 12.5 days (Woofter *et al.*, 2005). 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 brevetoxin elimination being slower in these species as compared to mammals (Nagy *et al*., 1999). However, it was interesting that Kemp's ridley and green turtles appeared to clear the toxin faster than loggerhead turtles and have a higher success rate during rehabilitation. The reason for this species difference in survivability may be due to differences in body mass and distribution of the toxin between species or in differences of diet and initial toxin load. Although this preliminary data suggests there may be species differences in the clearance of brevetoxin between loggerheads and other sea turtle species, the samples sizes for Kemp's ridley and green turtles were small. Additional data needs to be collected so potential species differences can be more meaningfully assessed.

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. This can be explained by the continued clearance of the toxin by the turtles while undergoing rehabilitation. However, in 100% of animals (n=29) that died during rehabilitation, brevetoxin was present in tissues or feces regardless of the duration of rehabilitation.

For animals that were examined histologically there were no definitive lesions associated with brevetoxin exposure. This is not surprising since many toxins, particularly the sodium-channel neurotoxins, do not cause pathologic lesions in some animals. Unlike some harmful algal toxins, such as domoic acid (Scholin *et al.,* 2000), brevetoxins may not cause specific pathologic changes in the brain. Brain lesions may only be apparent in animals chronically exposed to brevetoxin, the lesions may be dose dependent or may appear secondary to exposure to toxic metabolites. Acute doses of phycotoxins (usually neurotoxins) are considered to act quickly, and even though there are defined symptomatologies for specific toxins, there are usually few pathognomonic lesions to indicate exposure. However, because individual HAB species can produce multiple toxins and bioactive compounds; they can have different mechanisms, routes, and degrees of exposure; and they can vector and possibly facilitate disease by secondary pathogens, a complex suite of subtle pathological changes may be expected that can confound diagnostic interpretation. However, systematic documentation of these changes associated with brevetoxin intoxication is currently lacking (Landsberg *et al.,* 2007). Due to the nature of our study this deficiency cannot be addressed without performing an experimental investigation with controlled dosing of brevetoxin, which is not possible in the species currently being studied.

Although brevetoxin specific pathologic lesions were not found it was interesting that 11 animals had evidence of pneumonia on histopathology. In five of these animals the pneumonia was due to aspiration, which may have been secondary to paralysis of muscles from exposure to brevetoxin. Additionally, six brevetoxin positive animals that died or were dead stranded had secondary human interactions. Live turtles with brevetoxin exposure are generally reported as floating and unresponsive. These animals are comatose and unable to move their flippers or submerge. Therefore the incidence of negative human interactions, particularly boat strike, may increase in stranded sea turtles during red tide events.

Several possibilities exist for the greater concentration of strandings in 2005 than in 2006. Strandings may have occurred as a result of local red tide conditions; however, red tide was severe all along the coast of central west Florida. Another possibility is that Mote's stranding program was so busy responding to the large number of strandings in their primary response area that they did not respond to strandings in further locations. The significance of weekly counts and monthly counts in

the stepwise linear regression suggests that sea turtles respond to both acute *K. brevis* conditions and chronic conditions. Additionally, a majority of the dead stranded sea turtles were recovered during periods of high red tide cell counts corresponding to acute conditions while live animals stranded during periods with lower cell counts during more chronic conditions after toxin could have been concentrated through the food web.

In conclusion, brevetoxin exposure was found in 66 of 73 (90%) dead and live sea turtles stranding during the 2005 and 2006 red tide events off the west coast of Florida. In the majority of these animals brevetoxin exposure was determined to have caused or contributed to the strandings. Although additional animals were not tested it can be assumed that a similar proportion of sea turtles that stranded during this time were also affected by brevetoxin intoxication. Findings from this study highlight the fact that the majority of the live and dead sea turtles sampled during the 2005 and 2006 red tide events were positive for brevetoxin indicating 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.

Table 1: Pre-mortem brevetoxin levels in fluids and tissues from live sea turtles analyzed by ELISA (n=49). [All values in ng/g (blood=ng/ml); NEG=None detected (LD<1 ng/ml for plasma and whole blood); LD=Detection limit]

ID	Admit Date	Year	Species	Whole Blood/Plasma	Outcome
ST 0513	3/20/2005	2005	Kemp's Ridley	3.1	DIED
ST 0516	4/4/2005	2005	Kemp's Ridley	**NEG**	RELEASED
ST 0517	4/14/2005	2005	Loggerhead	**NEG**	RELEASED
ST 0522	5/16/2005	2005	Loggerhead	19	DIED
ST 0530	6/4/2005	2005	Loggerhead	**NEG**	RELEASED
ST 0532	6/15/2005	2005	Loggerhead	**NEG**	RELEASED
ST 0549	7/3/2005	2005	Loggerhead	43	DIED
ST 0568	8/1/2005	2005	Kemp's Ridley	78.4	RELEASED
ST 0574	8/6/2005	2005	Kemp's Ridley	82	RELEASED
ST 0576	8/7/2005	2005	Loggerhead	75.3	DIED
ST 0587	8/10/2005	2005	Loggerhead	70	DIED
ST 0590	8/10/2005	2005	Loggerhead	23.5	DIED
ST 05100	8/13/2005	2005	Loggerhead	54.6	DIED
ST 05141	9/15/2005	2005	Loggerhead	25.2	DIED
ST 05142	9/16/2005	2005	Loggerhead	18.5	RELEASED
ST 05144	9/17/2005	2005	Kemp's Ridley	74	RELEASED
ST 05146	9/27/2005	2005	Loggerhead	88.8	DIED
ST 05151	10/9/2005	2005	Loggerhead	46.2	DIED
ST 05153	10/9/2005	2005	Green	4.1	RELEASED
ST 05158	10/12/2005	2005	Loggerhead	46.2	DIED
ST 05170	11/28/2005	2005	Loggerhead	7.6	RELEASED
ST 05174	12/27/2005	2005	Green	**NEG**	RELEASED
ST 0604	1/15/2006	2006	Loggerhead	5.6	RELEASED
ST 0606	1/27/2006	2006	Green	**NEG**	DIED
ST 0611	2/10/2006	2006	Green	**NEG**	DIED
ST 0612	2/14/2006	2006	Green	0.8	REHAB
ST 0614	3/13/2006	2006	Loggerhead	2.4	DIED
ST 0617	3/10/2006	2006	Green	**NEG**	DIED
ST 0619	3/22/2006	2006	Loggerhead	2.2	DIED
ST 0633	4/26/2006	2006	Loggerhead	0.7	RELEASED
ST 0634	4/27/2006	2006	Loggerhead	2.1	REHAB
ST 0635	5/3/2006	2006	Loggerhead	2.1	REHAB
ST 0636	5/7/2006	2006	Loggerhead	**NEG**	REHAB
ST 0658	8/14/2006	2006	Loggerhead	3.5	DIED
ST 0666	8/25/2006	2006	Loggerhead	56.1	DIED
ST 0675	8/28/2006	2006	Loggerhead	62.7	DIED
ST 0678	8/30/2006	2006	Loggerhead	3.9	RELEASED
ST 0685	9/3/2006	2006	Loggerhead	4.2	RELEASED
ST 0686	9/4/2006	2006	Loggerhead	78.6	RELEASED
ST 0694	9/8/2006	2006	Loggerhead	73.8	RELEASED
ST 06102	9/18/2006	2006	Loggerhead	15.9	DIED
ST 06106	9/19/2006	2006	Loggerhead	30.2	DIED
ST 06129	9/30/2006	2006	Loggerhead	106.6	RELEASED
ST 06132	10/21/2006	2006	Loggerhead	80.4	RELEASED
ST 06133	10/22/2006	2006	Loggerhead	50.3	DIED
ST 06134	10/22/2006	2006	Loggerhead	40.6	RELEASED
ST 06136	10/28/2006	2006	Kemp's Ridley	82.3	RELEASED
ST 06137	11/5/2006	2006	Green	2.4	DIED
ST 06140	11/11/2006	2006	Loggerhead	7.1	DIED

Table 2: Brevetoxin levels in fluids and tissues from dead stranded turtles and turtles that died during rehabilitation analyzed by ELISA (n=46).

[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, St Cnts=Stomach Contents, LI Fluid=large intestine fluid; SI Fluid=small intestine fluid]

ST ID	Admit Date	Nec Date	Days In Rehab	Species	Dead Admit	Lung	Liver	Kidney	St Cnts	Feces	Bile	Urine	SI Fluid	LI Fluid	Admit Blood
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	27.6	NEG	16.8	21.9	*		*	*	*
ST 0538	6/18/2005	6/19/2005	0	LK	D	93.7	143.1	91.1	113.1	156.8	*		*	154.1	*
ST 0542	6/21/2005	6/22/2005	0	LK	D	89.0	397.2	105.0	593.0	649.7	*		*	*	*
ST 0566	7/31/2005	7/31/2005	0	LK	D	174.0	293.6	142.3	782.6	231.5	*		*	*	*
ST 0567	8/1/2005	8/2/2005	0	LK	D	185.6	564.1	348.8	1559.2	1045.6	*		*	*	*
ST 0575	8/6/2005	8/7/2005	0	LK	D	219.2	392.7	268.8	*	*	*		*	*	*
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	21.9	27.3	35.6	NEG	268.4	*		*	*	*
ST 0595	8/11/2005	8/11/2005	0	CC	D	NEG	53.3	33.5	26.7	33.6	*		*	*	*
ST 05103	8/13/2005	8/14/2005	0	LK	D	124.8	358.6	138.1	499.1	285.0	*		*	*	*
ST 0613	2/14/2006	2/14/2006	0	CM	D	*	NEG	NEG	NEG	*	*	*	84.9	NEG	*
ST 0626	4/16/2006	4/17/2006	0	LK	D	*	51.3	27.2	61.2	181.5	*	*	*	*	*
ST 0627	4/18/2006	4/19/2006	0	CC	D	*	7.5	9.8	NEG	15.7	50.0	8.4	*	*	*
ST 0637	5/8/2006	5/8/2006	0	CC	D	NEG	228.2	59.4	NEG	144.5	16.6	*	NEG	NEG	*
ST ID	**Admit Date**	**Nec Date**	**Days In Rehab**	**Species**	**Live Admit**	**Lung**	**Liver**	**Kidney**	**St Cnts**	**Feces**	**Bile**	**Urine**	**SI Fluid**	**LI Fluid**	**Admit Blood**
ST 0513	3/30/2005	6/2/2005	64	LK	L	NEG	NEG	NEG	*	22.8	*	*	*	*	3.1
ST 0522	5/16/2005	5/25/2005	9	CC	L	NEG	8.6	NEG	*	72.0	*	*	*	*	19
ST 0524	5/17/2005	5/19/2005	2	CM	L	NEG	NEG	NEG	NEG	70.0	*	*	*	*	*
ST 0549	7/3/2005	7/7/2005	4	CC	L	NEG	33.4	NEG	NEG	43.3	*	*	*	*	43
ST 0576	8/7/2005	8/9/2005	2	CC	L	31.9	74.8	76.2	224.1	*	*	*	*	*	75.3
ST 0587	8/10/2005	8/14/2005	4	CC	L	29.6	28.5	25.7	10.7	1007.0	140.4	*	*	*	70
ST 0590	8/10/2005	8/19/2005	9	CC	L	NEG	13.5	NEG	*	19.9	30.4	*	*	*	23.5
ST 05100	8/13/2005	8/17/2005	4	CC	L	NEG	*	10.0	24.2	184.9	10.0	*	23.0	10.0	54.6
ST 05116	8/18/2005	8/19/2005	1	CC	L	NEG	17.2	NEG	NEG	6.0	4.5	*	*	*	*
ST 05123	8/20/2005	8/30/2005	10	CC	L	NEG	NEG	NEG	*	13.5	NEG	*	NEG	NEG	*
ST 05124	8/20/2005	8/24/2005	4	CC	L	21.9	25.3	20.4	NEG	2012.1	33.9	*	20.2	417.5	*
ST 05125	8/21/2005	8/23/2005	2	CC	L	19.4	26.9	NEG	NEG	564.2	5.9	*	21.7	1178.5	*
ST 05141	9/15/2005	10/7/2005	22	CC	L	14.0	27.7	12.5	NEG	74.5	46.8	*	8.1	13.8	25.2
ST 05151	10/9/2005	10/13/2005	4	CC	L	9.1	16.4	13.9	308.0	1978.1	NEG	*	24.9	474.7	46.2
ST 05158	10/12/2005	12/8/2005	58	CC	L	NEG	NEG	NEG	*	28.6	23.9	*	6.4	29.2	46.2
ST 0606	1/27/2006	5/1/2006	96	CM	L	*	NEG	NEG	*	10.9	NEG	1.8	12.3	*	NEG
ST 0611	2/10/2006	2/11/2006	1	CM	L	NEG	NEG	NEG	NEG	46.7	14.1	NEG	71.0	NEG	NEG
ST 0614	3/13/2006	3/17/2006	4	CC	L	*	13.1	NEG	NEG	NEG	22.3	16.1	*	*	2.4
ST 0617	3/10/2006	3/24/2006	14	CM	L	*	NEG	NEG	NEG	NEG	23.1	*	*	*	NEG
ST 0619	3/21/2006	3/22/2006	1	CC	L	*	30.2	NEG	NEG	13.1	NEG	8.6	*	*	2.4
ST 0647	6/25/2006	6/25/2006	0	CC	L	*	13.2	NEG	NEG	NEG	NEG	NEG	NEG	NEG	*
ST 0666	8/25/2006	9/8/2006	14	CC	L	*	40.5	17.6	*	*	36.1	16.2	15.2	*	56.1
ST 0675	8/28/2006	11/1/2006	64	CC	L	*	14.5	NEG	*	*	11.6	10.4	*	*	62.7
ST 06102	9/18/2006	9/26/2006	8	CC	L	*	31.1	13.2	306.0	9.1	27.0	3.3	NEG	*	15.9
ST 06106	9/19/2006	10/12/2006	23	CC	L	*	16.2	8.9	*	*	NEG	*	*	*	30.2
ST 06133	10/22/2006	10/24/2006	2	CC	L	*	32.9	30.7	270.2	1902.2	143.5	*	243.1	*	39.2
ST 06137	11/5/2006	11/8/2006	3	CM	L	*	NEG	NEG	NEG	381.2	19.2	*	22.3	*	2.4
ST 06138	11/8/2006	11/9/2006	1	CC	L	*	469.3	137.3	11803.9	*	427.3	*	2117.4	*	*
ST 06140	11/11/2006	12/9/2006	28	CC	L	*	7.7	NEG	*	*	26.4	10.1	NEG	*	7.1

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	90.48	173.91	98.96	303.97	375.87	33.31	42.46	51.37
Stdev	81.87	177.81	109.54	468.23	526.24	23.66	60.05	88.97
N	14	17	17	16	13	2	2	3

Dead Rehab	Lung	Liver	Kidney	St Cnts	Feces	Bile	SI Fluid	LI Fluid	Urine	Admit Blood
Mean	7.87	33.61	12.63	681.43	367.83	43.60	161.60	235.97	7.39	28.39
Stdev	11.66	87.05	28.70	2695.90	674.26	90.10	524.89	401.33	6.42	25.21
N	16	28	29	19	23	24	16	9	9	22

Table 4: Percent of dead stranded turtles and turtles that died during rehabilitation positive for brevetoxin by tissue type

[St Cnts=Stomach Contents, LI Fluid=large intestine fluid; SI Fluid=small intestine fluid]

Dead Stranded				**Live Stranded**		
Tissue/Fluid	**% Positive**	**N**		**Tissue/Fluid**	**% Positive**	**N**
Feces	***100.00%***	***13***		***Feces***	***86.96%***	***23***
Bile	***100.00%***	***2***		Bile	75.00%	24
Liver	88.24%	17		Liver	71.43%	28
Kidney	82.35%	17		Kidney	37.93%	29
Lung	78.57%	14		Lung	37.50%	16
Stomach Contents	62.50%	16		Stomach Contents	36.84%	19
SI Fluid	50.00%	2		SI Fluid	75.00%	16
LI Fluid	33.00%	3		LI Fluid	66.67%	9
				Urine	77.78%	9

TABLE 5

Results of stepwise linear regression modeling

VARIABLE	INCLUDED IN MODEL?	MODEL COEFFICIENT (BETA)	P-VALUE
Ave cell counts week	Yes	0.00000006	0.020
Ave cell counts week prior	No	-.065	0.345
Ave cell counts month	Yes	0.0000001	0.005
Ave cell counts 2 months	No	0.127	0.364
Ave cell counts 6 months	No	-0.028	0.782
constant	Yes	1.139	0.000

Figure 1: 2005 Sea turtle strandings (n=174)

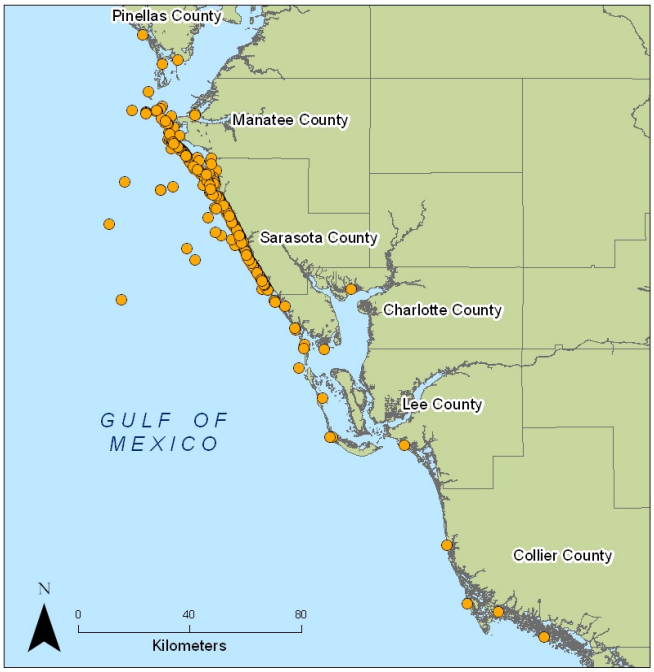


Figure 2: 2006 Sea turtle strandings (n=144)

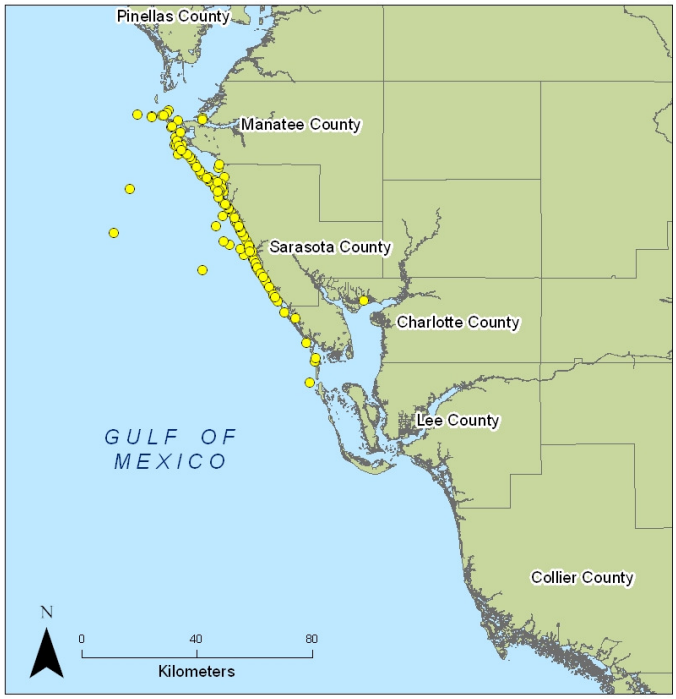


Figure 3: Clearance of brevetoxin from plasma in naturally exposed loggerhead turtles (n=19)
[Released animals highlighted in red triangles; animals that died highlighted with black squares]

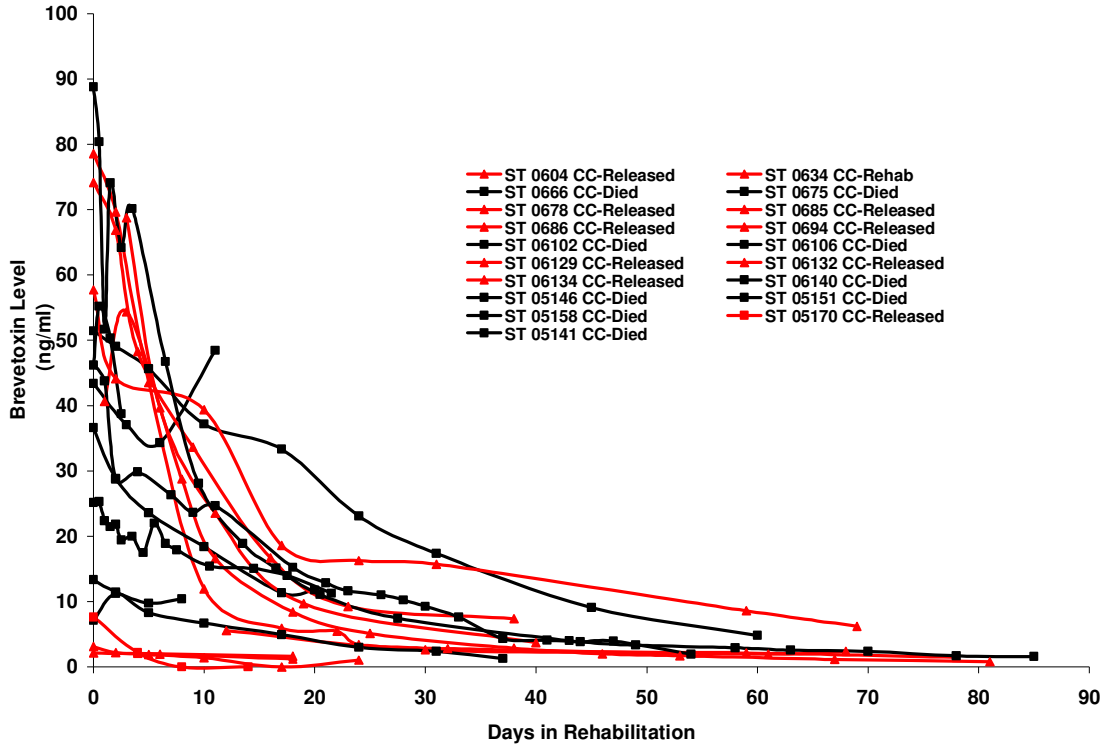


Figure 4: Clearance of brevetoxin in a naturally exposed Kemp's ridley and green turtles (n=3)
[Kemp's ridleys highlighted in red triangles; green highlighted with blue squares]

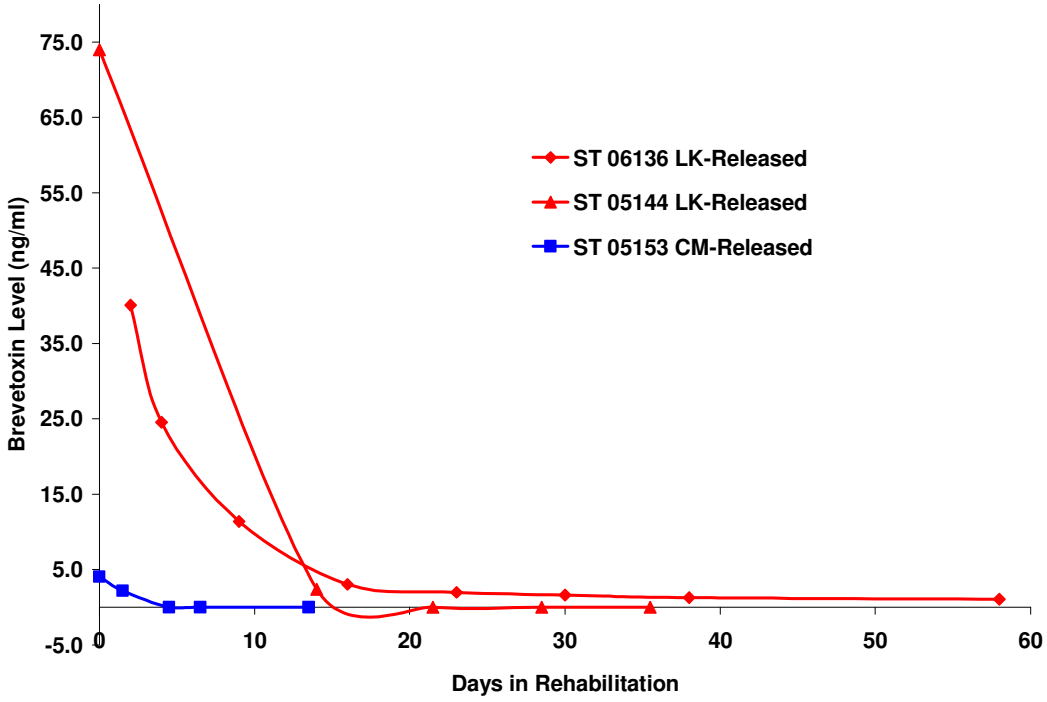


Figure 5: Percent elimination of brevetoxin from plasma after natural exposure in sea turtles (n=19)

[Released animals highlighted in purple or red triangles or diamonds; animals that died highlighted with black squares]

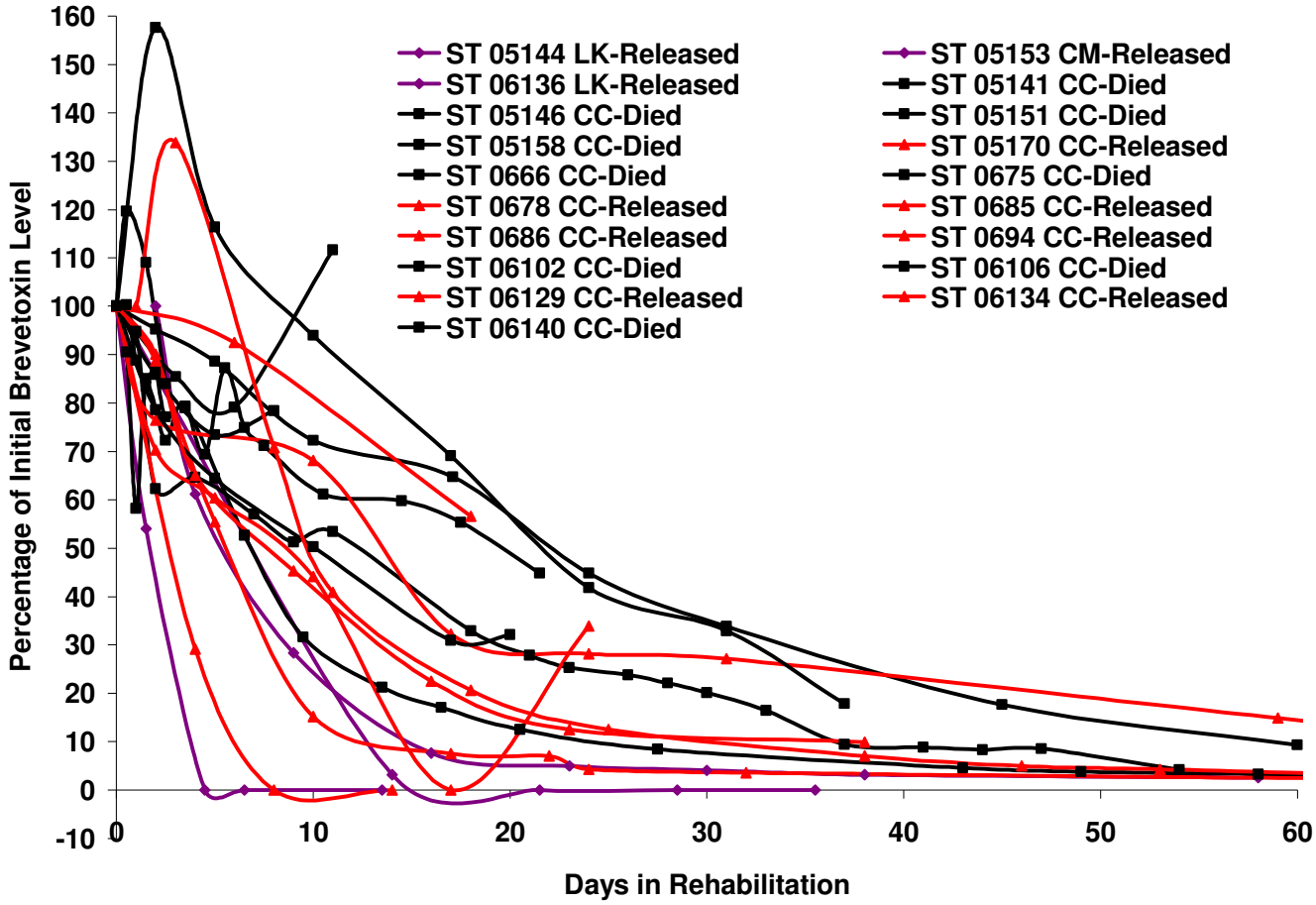
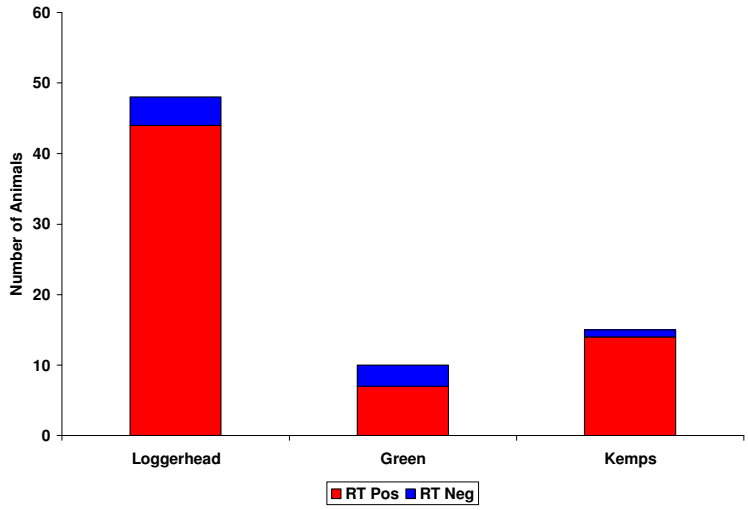
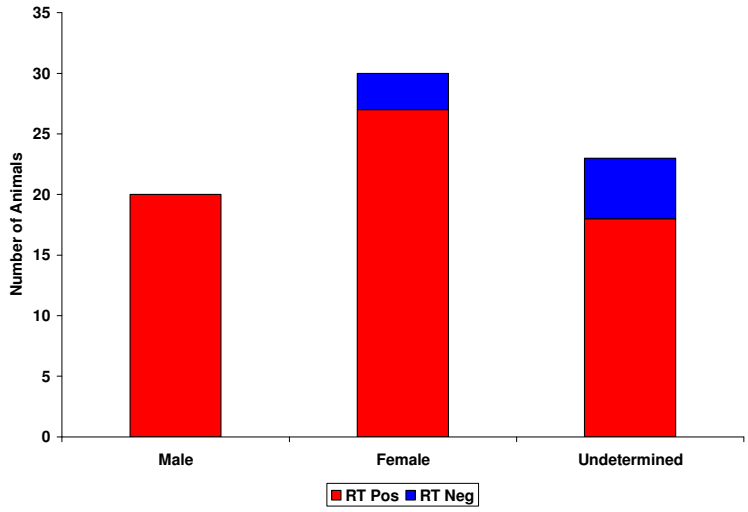


Figure 6: Proportion of red-tide positive turtles by species, sex, and season (n=73)





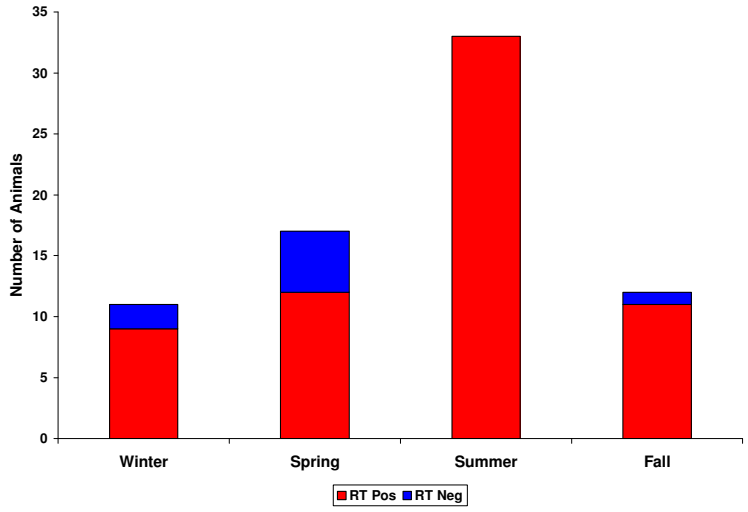


Figure 7: *K. brevis* cell counts at New Pass and City Island for 2005-2006.

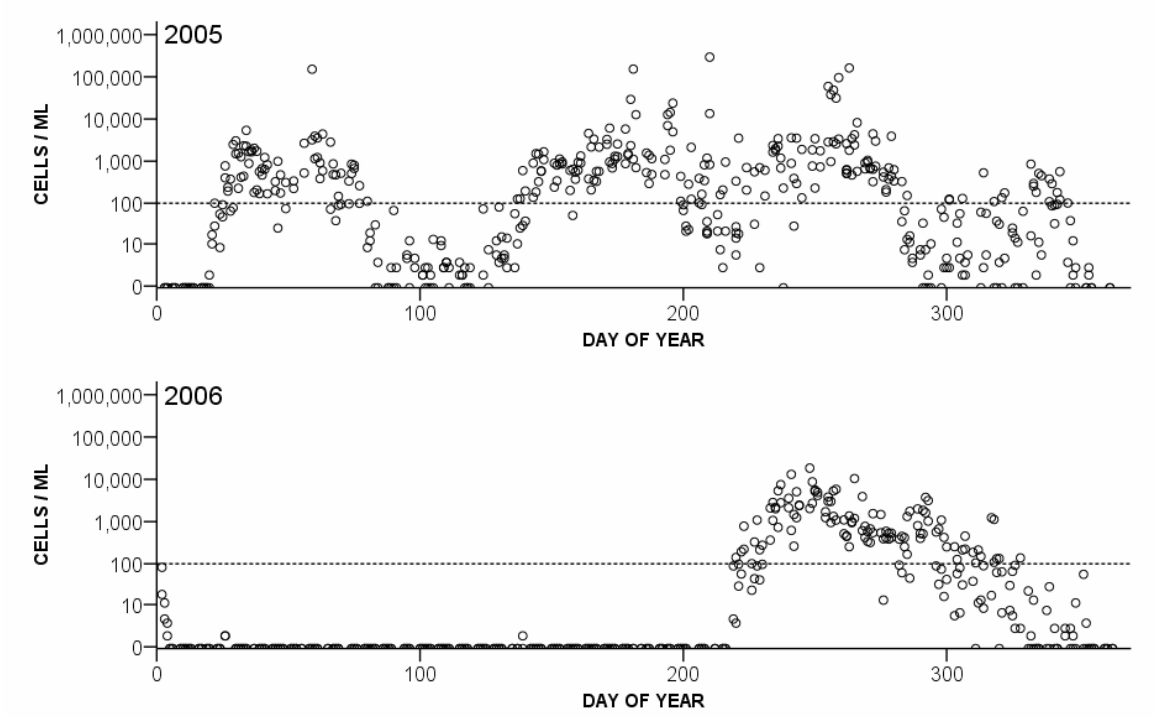


Figure 8: 2005 Sea turtle strandings by month (n=174)

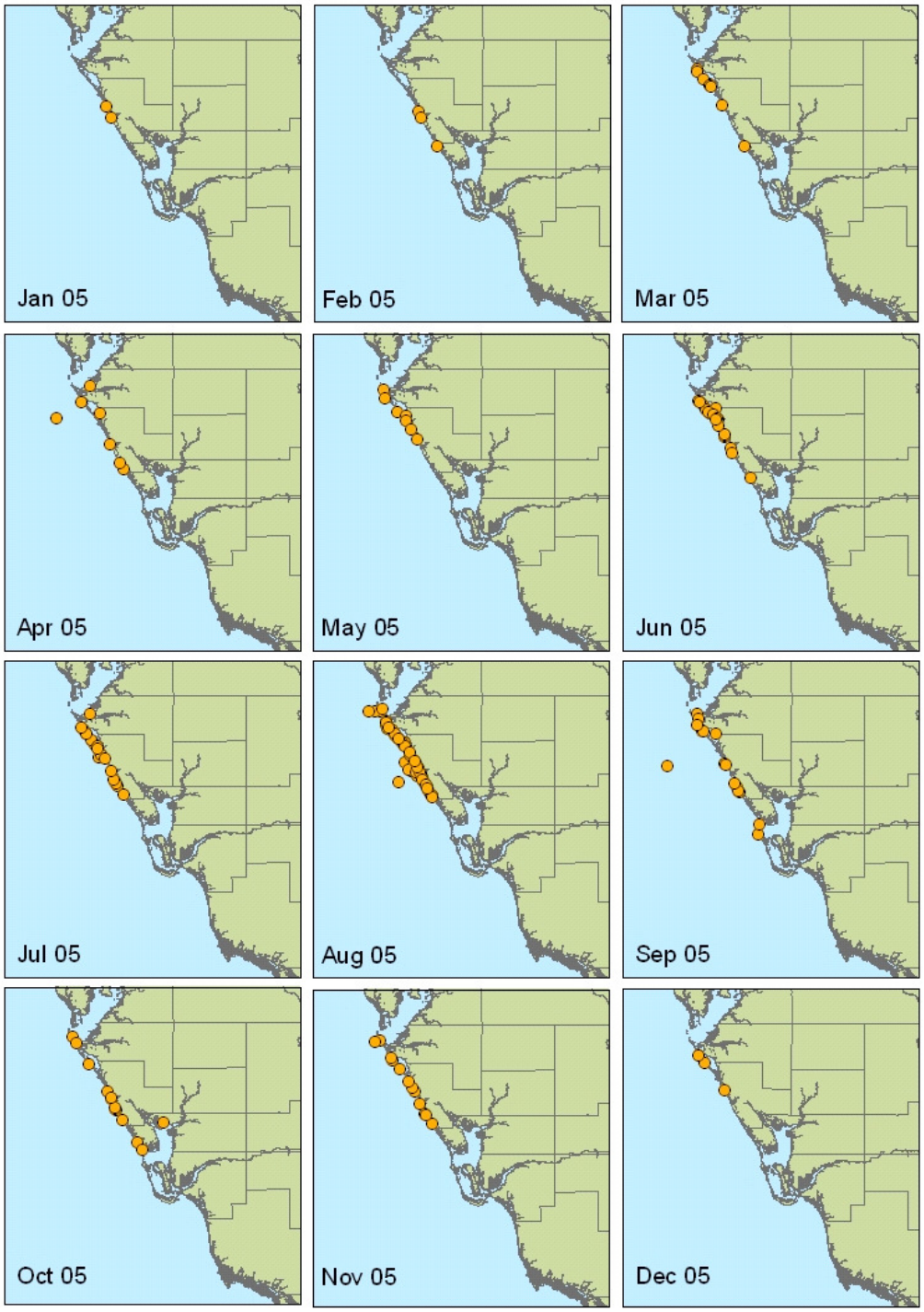


Figure 9: 2006 Sea turtle strandings by month (n=144)

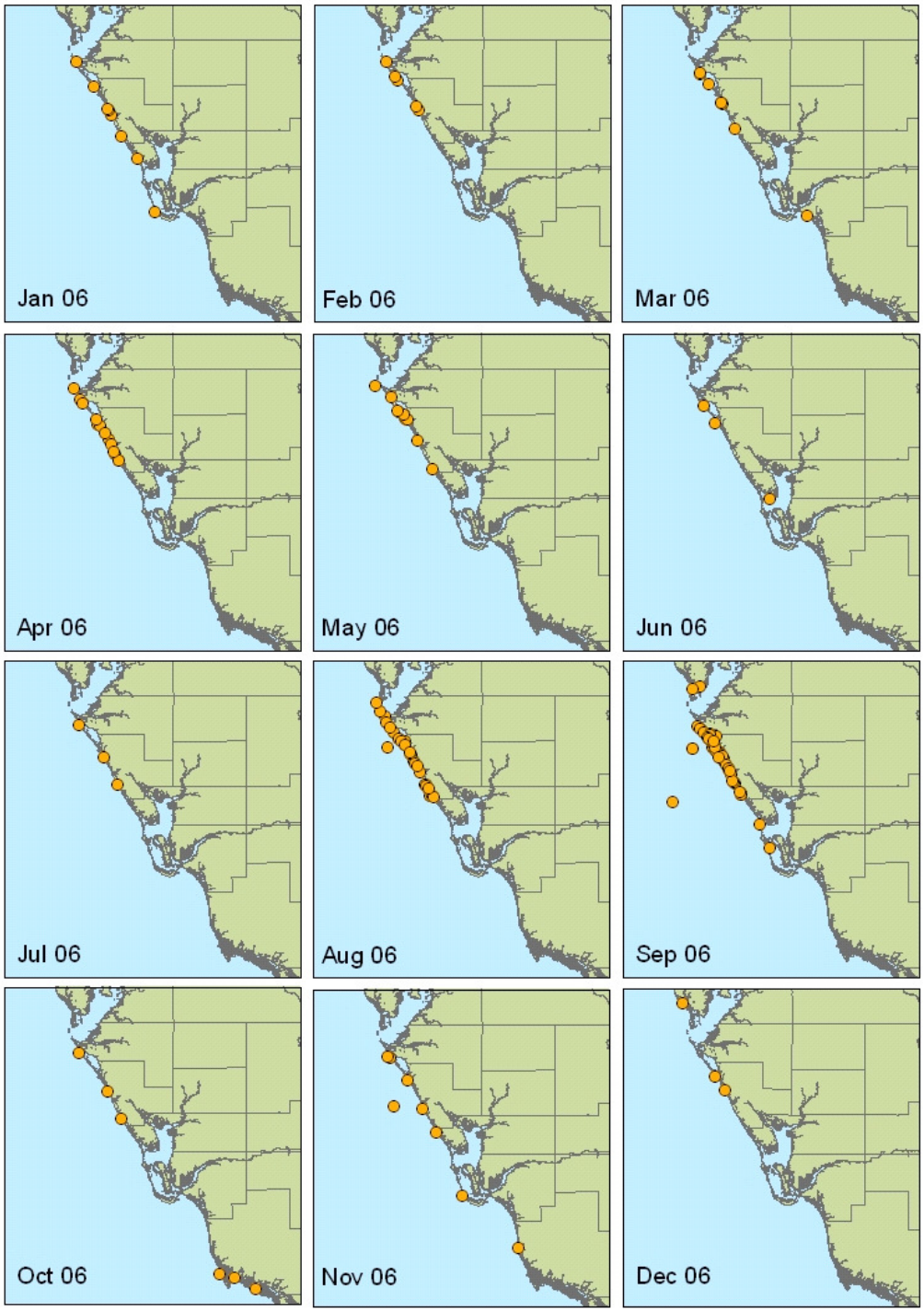
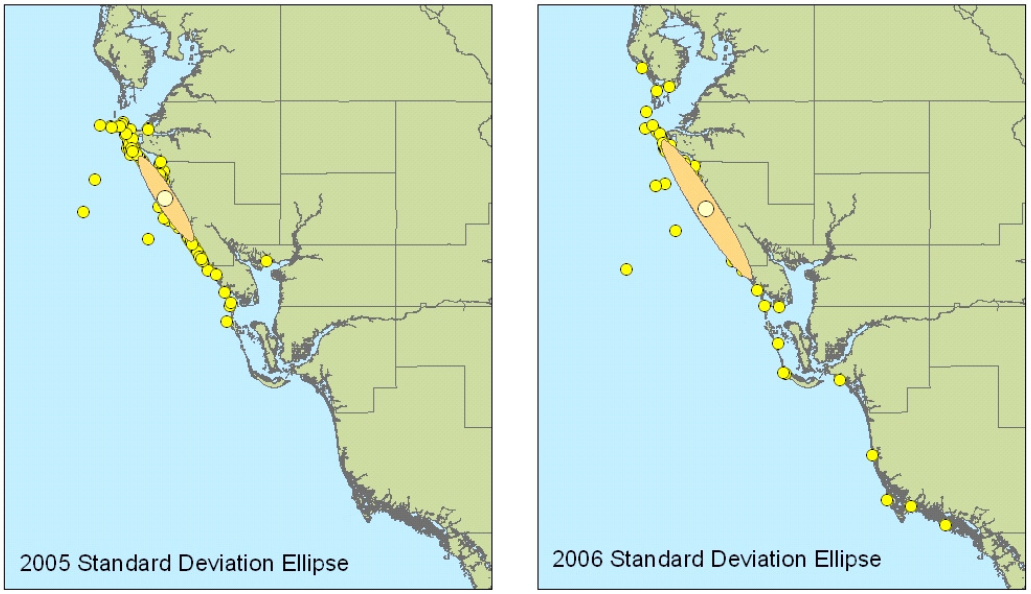


Figure 10: Deviation ellipses for 2005 and 2006 sea turtle strandings.



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