LOGGERHEAD NEUROLOGICAL DISEASE COMPLEX (2001 AND 2006 MORTALITY EVENTS) AND EVENT RESPONSE

INITIAL MEETING OF THE LNDC WORKING GROUP
MARCH 31/APRIL 1, 2008
HOSTED BY ST. CATHERINES ISLAND FOUNDATION

Participants, agenda items, event comparison and synopsis,
investigation needs, priorities, and protocols

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WORKSHOP PURPOSE AND SUMMARY

In the fall of 2006, a large number of loggerhead turtles stranded on the coasts of Georgia and northeastern Florida. Many affected animals were in good nutrition condition and presented with profound depression, elevated glucose levels, and extremely slow heart and respiratory rates. Extensive diagnostic testing failed to reveal the cause of this condition. Treatment was only successful in a limited number of cases. Turtles with similar clinical signs are still sporadically reported. The goal of the workshop was to bring together a group of experts and stranding network participants to discuss this mortality event and to initiate the creation of guidelines and recommendations for response to future mortality events occurring in the southeastern US. An emphasis was placed on problems that primarily manifest as neurological disease in loggerhead turtles, which is descriptively referred to herein as the loggerhead neurological disease complex. It is anticipated that this workshop is the first of additional such collaborative meetings, thus a number of future participants were identified.

The meeting was held March 31 and April 1, 2008 on St. Catherines Island. Summaries of the 2006 mortality event were given and comparisons with previous mortality events were discussed. Information collected during the event was presented, including stranding data, clinical and pathology data, results of algal toxin testing and environmental studies, and field observations. Various aspects of response and investigation were discussed to identify a prioritized list of aspects that need to be organized and incorporated into a rational investigative and management approach. The areas identified included the following: standardized protocol development; clinical and pathological observations that require more targeted study; potential for in-water collections during an event; harmful algal bloom studies, including options for screening unidentified biotoxins and field sampling; and the need to assess cost analysis and funding. Participants were tasked with obtaining relevant missing or unavailable data, including that pertaining to previous mortality events, as well as formulating methods and protocols.

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POTENTIAL FUTURE PARTICIPANTS

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Marine Science Center
Mote Marine Laboratory
Clearwater Aquarium
Marine Life Center
The Turtle Hospital on Marathon Key
Sea World Orlando
South Carolina Aquarium
University of Florida College of Veterinary Medicine

*These lists include individuals, facilities, and institutions that have participated in investigation/rehabilitation of animals involved in prior events and is not intended as an exclusive list of participants. Any individual or organization that would like to be involved should contact one of the active participants.

SUMMARY OF THE 2006 SEA TURTLE MORTALITY EVENT AND PAST EVENTS

2006 mortality event

A mass sea turtle stranding event was documented in Georgia and northeastern Florida in September and October of 2006. In Florida, the event involved the three northernmost counties, including Nassau, Duval, and St. Johns. In these counties, 83 strandings were documented in September, whereas the ten-year average was only eight. In October, 27 additional strandings were documented as compared to the previous ten-year average of nine. There were two primary pulses of strandings during these months. The first included the largest number of turtles (77 strandings) and was from September 8th through September 19th. The second group was from September 30th through October 10th, and included 19 strandings. Almost all (101) of the turtles were loggerheads. There were also five green turtles, two Kemp's ridleys, and two unidentified turtles. The mean straight carapace length of stranded loggerheads was 73.8 cm (SD = 8.0). A histogram illustrating frequencies of size classes is shown in Figure 1. Similarly, in Georgia strandings were documented during September 4th through October 15th from Sapelo Island to Cumberland Island (majority) (Camden and Glynn counties). The species composition was almost exclusively loggerheads, which included twenty-eight turtles. No significant associated mortality of other marine species was observed, nor was there any evidence of harmful algal bloom formation. The only environmental observation in the area was a *Trichodesmium* bloom.

Turtles presented with similar clinical signs, including profound depression, bradycardia, hypoventilation, and elevated blood glucose concentrations. Most animals were in fair to relatively good nutritional condition at the time of stranding. Several necropsied turtles had severe aspiration pneumonia. Clinical treatment was only successful in a small number of turtles. The etiology was not identified despite extensive diagnostic testing, including complete necropsy examination and biotoxin testing.

2001 mortality event

The 2001 loggerhead mortality event involved turtles that stranded in South Florida from Manatee County on the west coast to Palm Beach County on the East coast. Most turtles were found in the Florida Keys. This stranding event occurred from October 5, 2000 though March 2001, although 70% of strandings occurred during November through January. Only loggerheads appeared to be affected during this event. There were 189 strandings reported during this time period, which was six times higher than previous records. No mortality of other marine life was observed. The mean straight carapace length was 81.8 cm (range = 64.7-101.5). An extensive battery of diagnostic testing was performed including neurological examination, conduction studies, peripheral nerve biopsies, biotoxin testing, and various toxicological studies. There was evidence of a demyelinating neuropathy based on conduction abnormalities and peripheral nerve biopsies. The cause remains unknown. Neurological infection by spirorchiid trematodes (*Neospirorchis* sp.) was thought to be a potentially confounding health problem in some affected turtles.

2005/2006 brevetoxicosis-associated mortality event

Brevetoxicosis frequently occurs along the Florida Gulf Coast. In 2005 and 2006, brevetoxicosis events were especially notable because of significant associated sea turtle mortality. The affected area was concentrated from just north of Tampa Bay to the Florida Keys. Loggerheads, Kemp's ridleys, and green turtles were affected. Loggerheads, however, were more severely affected and took much longer to recover. In contrast to the unsolved 2001 and 2006 events, mortality of other marine life also was observed.

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RESULTS OF INVESTIGATION OF 2006 STRANDING EVENT

Clinical findings

All of the stranded loggerheads were assessed to be in good, fair, or thin body condition. None were observed to be emaciated. Body condition index as determined by simple length:weight ratio was compared between the mortality event turtles and those of live-capture turtles in Florida Bay and support that turtles affected during the event generally were mildly underweight (Figure 2). Also, it was anecdotally noted that nutritional condition declined as the event progressed; however, this was not supported based on stranding data collected in Florida. Similarly, epibiota and algae growth was observed to be greater in those turtles that stranded late in the course of the event.

Live stranded turtles that were brought into rehabilitation centers were severely depressed and weak, and many were characterized as comatose. On neurological examination, deep pain was absent peripherally (distal fore-and pelvic limbs), but present proximally and on lateral aspects of the cervical region. Reflexes and responses were depressed or absent. No voluntary movement was observed. Severe bradycardia (slow heart rate) was noted in most turtles, and was as low as less than 10 beats per minute (normal 30-35 bpm). Hypoventilation also was common finding and necessitated the use of artificial ventilation. Additionally, Michelle Bauer noted that several turtles could not open their mouths, as seen in a largely uncharacterized syndrome referred to in the rehabilitation community as “lockjaw.” None of the turtles examined at the University of Florida or in Georgia, which were tube-fed and examined by bronchoscopy, were observed to have this problem.

Turtles were treated with a combination of dry docking, fluid therapy, antibiotics, atropine (to stimulate heart rate), and artificial ventilation. No treatment was effective and most turtles eventually died or were euthanized due to welfare concerns.

Hematological and biochemical data

Blood work available for review from the 2006 UME consisted of values from11 animals, five from the University of Florida (UF) and six from the Marine Science Center (MSC) (Table 1). Unfortunately, comparison and interpretation are limited by the different laboratories, methodology used, and parameters analyzed. All blood analyses at UF were performed using an i-STAT, whereas MSC used Antech Diagnostics. Comparison of blood values with those of an ongoing study of wild loggerheads captured at the St. Lucie power plant is given in Table 1.

Six turtles presented with hyperglycemia and glucose values in five were only slightly elevated or were within the upper range of values seen in St. Lucie turtles. Five turtles had hypokalemia based on the St. Lucie values, which have a relatively narrow range compared to the generic chelonian values reported in reptile medicine texts (but similar to tortoise studies). An addition turtle had a marginally low potassium concentration and one turtle was hyperkalemic.

Ionized calcium was measured on two turtles and may have been low in both (based on some generic reptilian values - not included in St. Lucie data set), whereas total calcium was comparable to the St. Lucie values in the remaining turtles for which it was measured. Ionized

calcium concentration is poorly studied in sea turtles and requires further investigation to define normocalcemia.

Low hematocrit (not PCV) values are given for some of the UF turtles. These values are unreliable and should be disregarded. The other turtles had PCV's within the expected range, thus there is no evidence that anemia was a characteristic of this event.

The respiratory function, acid/base balance, and blood gases were followed in the UF turtles. Of the five UF turtles, two had low blood pH, elevated pCO2, and elevated HCO3 indicating partially compensated respiratory acidosis.

Necropsy findings

Necropsy findings from Florida turtles are given in Table 2. Nutritional condition ranged from good to poor based on adipose stores and muscle condition. Those turtles that were in poorer condition were in rehab for 10 to 14 days prior to necropsy. As noted in the clinical findings, none were characterized as truly emaciated. The most common finding was severe pneumonia (7/10 cases), which was most frequently associated with aspiration of sand and seawater. Infections by spirorchiid trematodes were relatively mild. There were no additional significant findings common in these cases. Histological findings largely supported gross findings. In summary, there was no evidence of trauma, specific organ injury (i.e. toxic injury), or significant primary inflammatory or infectious disease.

In addition, several turtles were noted to have intracoelomic hemorrhage. This finding was noted in turtles necropsied by T. Norton at the GADNR, SC Aquarium (received turtles from GA for rehab, field necropsies conducted by Carol Ruckdechel on Cumberland Island, and Charlie Manire from Mote Marine Laboratory. Hemorrhagic effusion also was noted in one turtle examined at UF. There was no correlative evidence of clotting abnormalities in live turtles nor was any source of hemorrhage identified in any case. The etiology and significance of this finding remains unknown.

Biotoxin testing

Samples from stranded turtles were tested for brevetoxin, domoic acid, saxitoxin, and okadoic acid by FWRI. All results were negative or interpreted as background. There was no correlative environmental data to further indicate the presence of these biotoxins. A *Trichodesmium* bloom was observed during the time of the event. Analysis for associated *Trichodesmium* toxin by LC-MS was performed in Dr. Peter Moeller's laboratory on water samples. No evidence of toxin was reported.

Non-biotoxin testing

The specifics of the event were discussed with Dr. Szabo's input as to whether additional toxicological testing of archived samples is indicated. It was decided that there were no specific findings with which to target toxicological studies. Cholinesterase inhibition assays may be incorporated into further studies. General guidelines for non-biotoxin testing are provided in Appendix V.

Miscellaneous additional tests

GC-MS

A sample of liver from one affected turtle was submitted the Michigan State Diagnostic Laboratory for GC-MS testing, which is performed to detect a broad range of pharmaceutical and agricultural toxicants. No suspicious organic compounds were observed.

Stable isotope analysis

Stable isotope analysis was performed on scute samples from the ten turtles necropsied at UF. Carbon values did not indicate a recent change in habitat and were consistent with an oceanic signature, either open ocean or edge of a coastal shelf. The newest scute layer suggested diet low in nitrogen, possibly jellyfish; however, evidence of benthic feeding (crab shell) was observed in the few turtles that had ingesta in their gastrointestinal tracts. Ideally, plasma would have been tested to provide a more proximate indicator of diet; however, adequate sample volume was not available.

Comparison with observations during previous mortality events

2001 South Florida event

Several findings were common to both the 2001 and 2006 events. Both events primarily involved turtles that stranded in relatively good nutritional condition with clinical signs that were characterized as severe depression and lethargy with diminished or absent reflexes and responses as assessed by neurological examination. Severe bradycardia also was observed in both events. Other findings in common included hyperglycemia and hypokalemia. Hypomagnesemia was reported during the 2000/01 event, but was not included among the data collected from the 2006 turtles. A significant number of turtles from both events had pneumonia as a confounding health problem. The prevalence and intensity of spirorchiid trematode infection observed in the 2000/01 event was not observed in turtles that stranded in 2006. In the Florida stranding data, turtles of smaller size classes (less than 85 cm) have a significantly lower prevalence of neurospirorchiidiasis (unpublished data – Stacy). The obvious missing comparative information is the advanced neurological evaluations performed in 2000/01, which were one of the most informative diagnostics used in that event. Conduction studies were performed on one of the affected turtles from the 2006 and were noted to be normal. No peripheral nerve biopsies were performed at that time, primarily based on lack of evidence of peripheral neuropathy. Unfortunately, retrospective evaluation of the results of conduction studies found that documentation of findings were inadequate (conduction velocities were not noted and specific interpretation was not provided). Efforts to obtain this information were unsuccessful. Therefore, the results of this evaluation are not regarded as reliable or necessarily comparable to the 2000/01 testing.

Brevetoxicosis physical exam findings

The general presentation of stranded loggerheads during the 2000/01 and 2006 events was similar to clinical signs associated with brevetoxicosis in that affected turtles are

profoundly depressed and lethargic, and in many instances were regarded as comatose. Signs observed with brevetoxin exposure that were not included in clinical signs noted in other events included conjunctival swelling, prolapsed cloaca and penis, and generalized edema. Further bradycardia was not a consistent finding with brevetoxicosis (pers comm C. Manire). Advanced neurological studies, including conduction studies and nerve biopsies have not been performed in cases of brevetoxicosis.

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AREAS OF IMPROVEMENT FOR INVESTIGATIVE RESPONSE AND PROTOCOL DEVELOPMENT

Overview

Retrospective review of findings from the 2006 event and comparison with previous events indicated that several areas of investigative and diagnostic approach need to be modified and, whenever possible, standardized. In addition, several options were proposed for field response and diagnostic testing. These aspects will be discussed separately as clinical and pathology studies, field response, and diagnostic testing.

Clinical and pathology studies

Lack of specific case definition, variability in minimum database collected, differences in the use of terminology, and differences in samples collected have significantly limited comparability between events, as well as, case data from the same event, and understanding of sporadic cases with apparently similar clinical signs. Discussions from this meeting highlighted several areas of needed improvement that fall under general mortality event investigation and specific disease study of the loggerhead neurological disease complex. These discussions included the following:

Mortality event response

1. Definition of unusual mortality event
2. Uniform case identification
3. Standardized antemortem and postmortem sample collection
4. Repository of clinical records, biological samples, and other data

Loggerhead neurological disease complex

1. Develop case definition
2. Standardize approach to physical examination and neurological examination
3. Enlist the expertise of a qualified neurologist for event response and insure that advanced neurological studies are performed
4. Better characterization of brevetoxin effects

Mortality event response

Definition of unusual mortality event

The following criteria have been adapted from those of the Working Group on Marine Mammal Unusual Mortality Events.

1. A marked increase in the magnitude or a marked change in the nature of morbidity, mortality or strandings when compared with prior records.
2. A temporal change in morbidity, mortality or stranding occurring.
3. A spatial change in morbidity, mortality or stranding occurring.

4. The species, age, or sex composition of the affected animals is different than that of animals that are normally affected.
5. Affected animals exhibit similar or unusual pathologic findings, behavior patterns, clinical signs, or general physical condition.
6. Morbidity is observed concurrent with or as part of an unexplained continual decline of a population, stock, or species.

Uniform case identification

The use of multiple forms of case identification has proved very problematic in the investigation of previous events, especially those turtles treated in rehabilitation centers. Some animals have as many as five different identifiers, including multiple common names. *The STSSN number is the most unique and broadly applicable identifier and should be used in the labeling of all biological samples, data sheets, and hospital records*. Names, permitting office numbers, and other identifiers should be noted along with the STSSN number to facilitate cross-referencing, but the STSSN number should be clearly indicated and should accompany records to secondary and tertiary rehabilitation centers.

Standardized antemortem and postmortem sample collection

A standardized live turtle and necropsy sampling protocol is provided in Appendices I-III. The necropsy protocol is prioritized and tiered in an effort to provide a realistic approach to consistent sampling during a mortality event. Of special concern is *blood collection upon admission to rehabilitation centers or other veterinary care facilities*. Blood samples from the immediate stranding interval are valuable and frequently are not collected or are of insufficient volume for necessary testing and archiving. Any medical personnel/facility that participates in the evaluation of mortality events in sea turtles is asked to adhere to this sampling protocol and complete the accompanying data sheets. In addition, the observation of hemorrhage within the coelomic cavity remains unresolved in terms of potential cause. Care should be taken to aspirate coelomic fluid (using a needle and syringe) prior to entering the coelomic cavity during necropsy to check for hemorrhage and to prevent misinterpretation of postmortem contamination of the coelomic fluid from severed blood vessels.

Hematological and biochemical analyses. Blood values obtained from different laboratories using different methodologies have limited comparability. Included in the protocol is the use of the University of Florida Clinical Pathology Laboratory for biochemical analysis on initial blood samples collected from live, stranded turtles. Rehabilitations centers should use their usual laboratories for biochemical values as necessary for clinical management, but analyses will be repeated at the UF laboratory. Again, only the initial blood sample prior to the initiation of treatment should be submitted to UF for biochemical analyses. Additional plasma samples should be stored frozen and submitted for archival storage with the collection date clearly indicated. Blood should be collected in lithium heparin and the plasma (3-6 mls) separated after centrifugation and shipped under refrigeration (cold pack) within 24 hours. Two unstained blood films also should be submitted to obtain an estimated differential white blood cell count. Samples should be shipped to:

To the attention of: Dr. Brian Stacy
University of Florida, College of Veterinary Medicine
2015 SW 16th Avenue
Room: VC-83
Gainesville, FL 32608
(352) 392-2212 ext. 5788

*Shipments should only be sent to UF on Monday through Thursday (couriers cannot deliver to the University on weekends). If initial blood collection is performed on Friday or on a weekend, collect and freeze plasma for toxicological testing, as indicated in the clinical sampling protocol, and submit a second plasma sample on the following Monday for biochemical analyses.

Repository of clinical records, biological samples, and other data

The University of Florida, College of Veterinary Medicine will serve as a central repository of samples and data collected during sea turtle mortality events and other relevant materials as needed. Facilities, including ultralow freezer storage, for this purpose have been provided by the National Marine Fisheries Service. A protocol for shipping of samples is provided in Appendix IX. The shipping address is as follows:

To the attention of: Dr. Brian Stacy
University of Florida, College of Veterinary Medicine
2015 SW 16th Avenue
Room: VC-83
Gainesville, FL 32608
(352) 392-2212 ext. 5788

Please contact Dr. Stacy in advance of any shipment.

Loggerhead neurological disease complex investigation

Case Definition

Use of the terms “lethargic loggerhead syndrome” or “lethargic loggerhead” has become relatively common within the veterinary and rehabilitation communities. Application of these descriptive terms has been inconsistent and varies between individuals to the extent that it is largely uninformative with regard to identifying cases with clinical signs comparable to the 2000/01 and 2006 mortality events. Chronically ill, emaciated turtles and animals with a variety of problems may be characterized as “lethargic,” thus creating confusion among biologists, rehabilitators, and veterinarians. Better understanding of clinical signs observed during these mortality events requires an evolving case definition that can be applied to appropriately identify cases, trigger application of data collection and sampling methods, and segregate similar sporadic cases from those with other problems. At this stage of limited understanding the case definition of is relatively broad and consists of the following criteria:

1. Loggerhead sea turtle
2. Nutritional condition is good or fair
3. Diminished reflexes and responses based on neurological examination
4. Bradycardia (less than 10 beats per minute)

Nutritional condition may be poor during the course of any event, but animals that are notably thin or chronically emaciated should not be considered in assessing sporadic cases to avoid inclusion of turtles with various problems that ultimately may result in depression or lethargy. Only cases that receive complete physical examination are to be included. Turtles that are spatially and temporally associated with a mass stranding event and are recovered dead or die prior to examination are regarded as probable cases. Further criteria for those that are necropsied include lack of gross or histopathological evidence of specific primary organ injury. Bacterial/aspiration pneumonia is not a criterion for inclusion or exclusion. In addition, hyperglycemia, which was a common finding in the turtles of both events, may be considered as supportive, but is not a criterion because it is not consistently observed and it appears to be relatively nonspecific in sea turtles. *Live stranded turtles that meet the above criteria should be reported to the state stranding coordinator, which will then contact the collaborating veterinary network.*

A potential confounding issue in sporadic cases, and potentially in stranding events, is near drowning due to forced submersion (e.g. fisheries interaction). Although this phenomena has not been studied, the anticipated clinical symptoms and necropsy findings may overlap considerably. Thus, caution must be applied, especially in the evaluation of sporadic cases. Observers should carefully examine animals for evidence of any entrapment event (entanglement wounds or abrasions). Advanced neurological methods, e.g. conduction studies, may be of value in distinguishing these cases.

Standardize approach to physical examination and neurological examination

Guidelines are provided for standardized physical exam and neurological exam (see attached PDF of article in JAVMA and draft form in Appendix I and II). Any live stranded turtle that falls within the case definition should be examined by a veterinarian from the working group, which should be expanded to include adequate coverage for rehabilitation centers.

Enlist the expertise of a qualified neurologist for event response and insure that advanced neurological studies are performed

Dr. Thomas Schubert, the chief of the neurology service at UFCVM, was previously involved with EMG studies on a few turtles during the 2000/01 event. He is interested in pursuing evaluation of these animals in the future and has a portable EMG unit that may be used if needed.

Better characterization of brevetoxin effects

Brevetoxin-associated mortality events represent one of the few known causes for mass stranding events involving sea turtles. Furthermore, brevetoxin produces similar neurological deficits to those observed during the 2000/01 and 2006 events. Characterization of nerve conduction abnormalities and ultrastructural changes in peripheral nerve biopsies associated brevetoxin exposure would be a valuable comparative reference for unsolved events. Representative affected turtles from the next brevetoxin-associated event will be examined to obtain this information.

Field response

Recommendations for field response to mortality events included efforts to capture live affected turtles by trawling and the need for means of collecting water samples for harmful algal bloom testing. The proposed trawler sampling of turtles is primarily for a repeat event in the north Florida, Georgia, South Carolina area where boats and personnel are available. A preliminary overview of costs associated with trawler collections and contact information is given in Appendix VIII. Samping protocols and permit applications have yet to be drafted. The South Carolina DNR and Georgia DNR are identified as resources for water sample collection, and other state agencies likely will have to be enlisted in the face an event. A protocol for water and environmental sampling for microalgal abundance and toxin analysis is given in Appendix V-VI.

Electronic database development (notes from D. Griffin)

Currently, the Marine Mammal Unusual Mortality Event Working Group and NOAA utilize an online system for management of nearly all data from an Unusual Mortality Event. This includes Level A data, Google maps (spatial data), pathology reports, gross necropsy reports, lab results, images, and any reports relating to the event. This data management system provides a mechanism for collaborative efforts at a regional level to study marine mammal health and provide an integrated approach to the management of an UME.

There is a need to develop a similar, if not identical, collaborative sea turtle health data management system. There are two possible approaches to this. The first is to use the same system that is being used for marine mammals. If this route is taken (with the proper approvals-Dr. David Rotstein and Dr. Teri Rowles), there still is the question as to where this system will be housed. It will need its own computer server that can execute the particular operating system/platform/language and can also provide the necessary space for storing the data. At this time, I (D. Griffin) am unable to estimate or predict what funds would be needed to follow this route.

The other option is to develop, from the ground up, a sea turtle data management system. This will require hiring a developer to build the web based system. I have spoken with Michael Coyne of seaturtle.org concerning this and he is interested in the project. He has

already built a web based system that allows us to enter our stranding data (http://www.seaturtle.org/strand/). This tool includes google maps, quality control error checking and data summaries, both spatial and temporal. It is very similar to the Level A data section of the marine mammal web site. It is possible to take this a step further to develop a system that is similar to that described above. If this route is taken, we still need to address the server issue mentioned above as well as funds to compensate the developer.

Once a system is up and running, the final decision to be made is whether or not all sea turtle cases and necropsy data are entered into this data management system or only a selected group of them. My recommendation is that since we only know a small amount about sea turtle health, it would be a better comprehensive approach to include all the data that is properly collected using the developed protocol. There is one other issue that is noteworthy. Currently, every state is managing their Level A stranding data in different ways. South and North Carolina use http://www.seaturtle.org/strand/. I am not familiar enough with how Florida, Georgia or the rest of the Eastern, Pacific and Gulf States manage their stranding data. Furthermore, the SEFSC Miami lab has just built yet another online system that is incompatible with other online systems. Therefore, regardless of what route is chosen, data managers from all states of interest (southeast?) and the appropriate federal partners need to be included in the development of this system. Guidance from the NMFS and USFWS National Sea Turtle Coordinators should also have input. This will only improve its chance of success into the future.

There have been mixed feelings about which system to use. Some initial work has already been started with SC and NC and Michael Coyne and it may be good to take this direction because a lot of work has gone into it and it appears to be working very well.

Additional suggestions and studies

The following items were suggested for further development:

1. Identification and development of addition techniques for additional biotoxin testing
    a. Develop methods for BMAA testing
    b. Consult with Peter Moeller for methods of detecting unknowns
    c. Obtain water samples during 2008 and/or 2009 in-water surveys for HAB testing

2. Use of i-stats at rehabilitation centers to monitor blood gases and ionized calcium

3. Study of epibiont and algae accumulation in the fall in GA/NE FL

4. Develop method for collecting cerebrospinal fluid (CSF) from live turtles for analysis

BULLETED RECOMMENDATIONS FOR FUTURE INVESTIGATIONS

Mortality event response

1. Adopt criteria for unusual mortality events in sea turtles similar to those applied for marine mammals with appropriate modification.

2. Incorporate standardized clinical and necropsy examination protocols (see appendices) with specific recommendations on data and sample collection.

3. Perform hematological and biochemical analyses at University of Florida, College of Veterinary Medicine to promote comparability between cases and application of in-house reference intervals.

4. Apply protocols for biotoxin testing, including environmental sampling, prey items, clinical cases, and necropsy samples (see appendices).

5. Archive biological samples and other data at the University of Florida, College of Veterinary Medicine for future availability.

6. Develop electronic database for health and disease related data and mortality event investigation.

7. Develop protocols for trawler sampling to recover affected turtles during event.

Loggerhead neurological disease complex

1. Utilize case definition of loggerhead neurological disease complex to recognize future cases and trigger necessary data/sample collection.

2. Until this condition(s) is better characterized, include diagnostic study of cases of sporadic mortality that fit the case definition.

3. Include advanced neurological examination techniques (conduction testing and peripheral nerve biopsy) in minimum database from a representative subset of affected turtles in collaboration with neurologist(s).

4. Perform advanced neurological examination, including conduction studies and peripheral nerve biopsies, on symptomatic turtles exposed to brevetoxin for comparative study.

Table 1. Hematological and biochemical vales for stranded loggerhead turtles during the 2006 mortality event.

STSSN#	UF#	Name	Necropsy#	HCT/PCV (%)	WBC (10^3)	T Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	AST (U/L)	CK (U/L)	UA (mg/dl)	Na (mEq/L)
DBH2006091602	#1	Pledes	N06-69	15	-	-	-	-	-	-	-	147
AMF2006091501	200786	Theodore	N06-626	14.6	7	2	<1.0		426	1748	1	150
DBH2006091601	#3	Coral		19	-	-	-	-	-	-	-	150
MPD2006091601	#4	Tort		38	-	-	-	-	-	-	-	140
AMF2006091601	#2	Mickey	N06-68	<10	-	-	-	-	-	-	-	155
MSC turtles	MSC #	Name										
	CC0648	Bugga		27	9	5.3	1	4.3	152	925	0.3	150
	CC0649	Flo		29	9	4.9	1.1	3.8	147	579	0.5	157
	CC0650	A1		36	11	5.7	1.6	4.1	215	2103	0.6	156
	CC0651	Alberta		26	8	5	1.1	3.9	140	450	0.6	147
	CC0652	Cubbie		28	5	4.4	1	3.4	161	271	1.6	154
	CC0657	Olga		34	7	6.4	1.3	5.1	135	1392	0.5	151
Archie Carr Center	Mean			28.3	-	3.37	1.2	-	249	927	0.85	157
(St. Lucie values)	Ranges			17-33.7	-	2.6-4.1	0.8-3.1	-	169-345	319-1742	0.6-1.4	150-168

STSSN#	UF#	K (mEq/L)	Cl (mEq/L)	BUN (mg/dl)	Ca (mg/dl)	P mg/dl)	Glucose (mg/dl)	pH	pCO2	O2	HCO3	TCO2
DBH2006091602	#1	2.7	112	62	ionized-0.8	150	7.97	18.2	300	42.3	43	-
AMF2006091501	200786	3.5			7.1	5.3	182	7.46	55.9	158	39.7	41
DBH2006091601	#3	3.2	120	50	ionized-0.97	182	7.29	60.4	98	28.8	31	-
MPD2006091601	#4	2.5	108	20	-	-	285	7.3	76	74	37.1	39
AMF2006091601	#2	2.8	110	57	-	-	110	7.45	61.4	73	42.4	44
								7.5-7.7	29-42		20-30	
MSC turtles	MSC #											
	CC0648	4.4	107	35	6.5	8.7	295	-	-	-	-	-
	CC0649	4.5	118	30	7	9.5	99	-	-	-	-	-
	CC0650	3.9	98	81	6.9	8.4	114	-	-	-	-	-
	CC0651	6.1	110	70	6.7	10.1	167	-	-	-	-	-
	CC0652	4.8	114	54	6.1	8	174	-	-	-	-	-
	CC0657	2.5	107	13	8.9	7.2	234	-	-	-	-	-
Archie Carr Center	Mean	3.9	113	50	6.16	7.63	95	-	-	-	-	-
Values	Ranges	3.5-4.4	103-126	36-74	4.3-7.1	6.3-9.5	73-111	-	-	-	-	-

Table 2. Necropsy findings in stranded loggerhead turtles during the 2006 mortality event.

Case*	Body condition	Pneumonia	Spirorchiids	Other
Complete necropsies				
1. (5 days)	Thin	Yes, severe aspiration	Incidental, none in CNS	Epicarditis
2. (8 days)	Thin	Yes, mild	Incidental, none in CNS	
3. (9 days)	Thin	Yes, severe	Arteritis with thrombi, none in CNS	Possible melena
4. (10 days)	Poor	Yes, severe aspiration	Incidental, rare in CNS	Nematodiasis (large)
5. (6 days)	Thin	No	Incidental, rare in CNS	
6. (14 days)	Poor	Yes, severe aspiration	Incidental, rare in CNS	Herpetic dermatitis
Gross necropsies				
7. (0 days)	Good	No	Incidental, none in CNS	
8. (0 days)	Thin	No	Incidental, few in CNS	
9. (0 days)	Thin	Yes, severe aspiration	Incidental, rare in CNS	
10 (0 days)	Good	Yes, severe aspiration	Incidental, none in CNS	

*Numbers in parenthesis are days post-stranding.

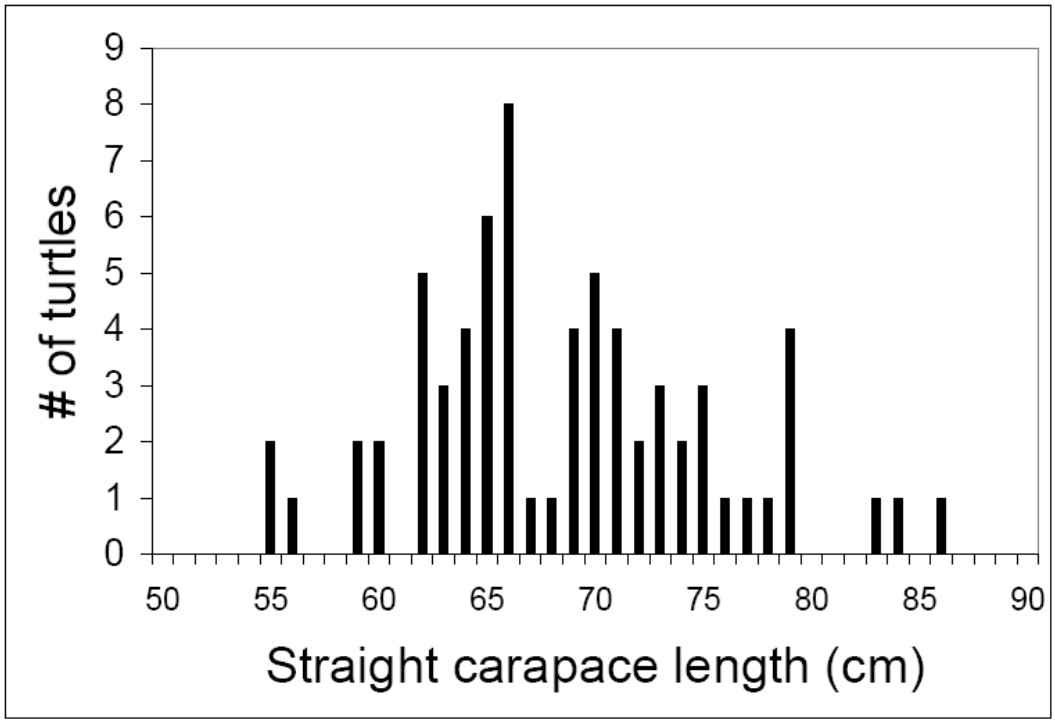


Figure 1. Histogram of frequencies of straight carapace lengths of turtles stranding during the 2006 loggerhead mortality event.

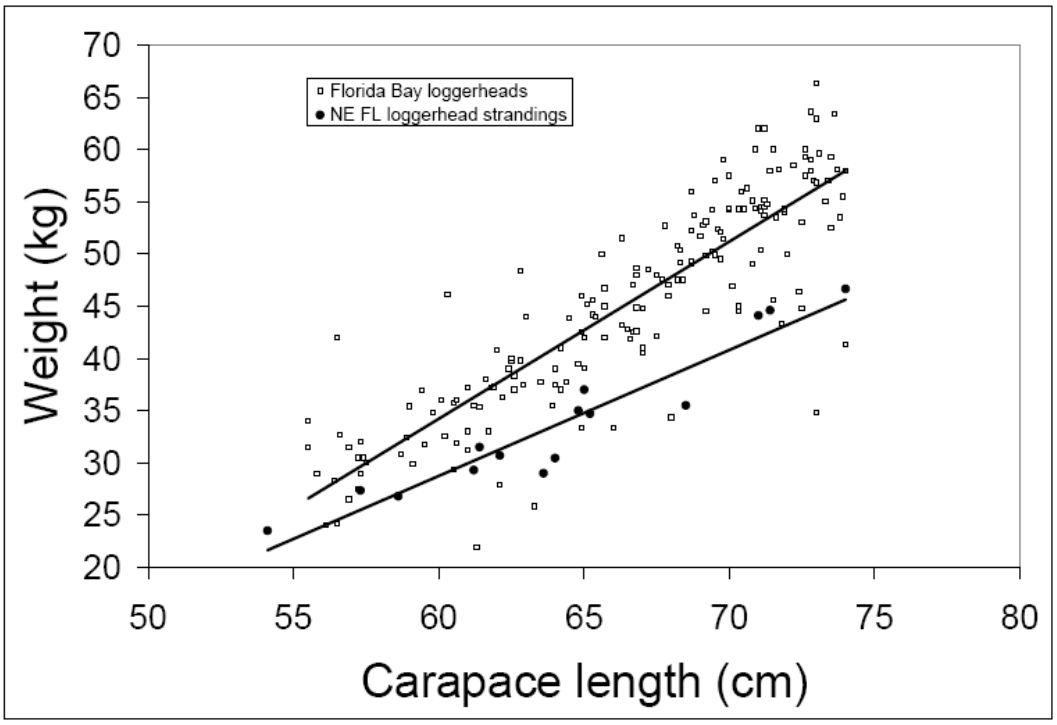


Figure 2. A comparison of the length vs. weight of the stranded turtles when first recovered with that of similar-sized, wild loggerheads captured in Florida Bay shows that the stranded loggerheads were mildly underweight relative to apparently healthy turtles (A. Foley).

Figure 3. Epibiota accumulation and algal growth was minimal during the early phase of the 2006 event (left image), but was frequently observed in those that stranded later (right image).

APPENDIX I
LOGGERHEAD NEUROLOGICAL DISEASE COMPLEX LIVE TURTLE EXAMINATION FORM

STSSN NUMBER: _____ DATE OF EXAM:_____

VETERINARIAN OR REHABILITOR PERFORMING EVALUATION
First _____ M.I.____ Last _____
Affiliation _____
Address _____
_____
Area code/Phone number _____

PHYSICAL EXAM:

Weight actual / est. **_____** kg / lb (obtain actual weight if possible)

Note: Normal=N, Abnormal=A, abnormalities are described in clinical notes and diagram section below

Attitude: N □ A □, **Activity:** N □ A □, **Can turtle go in water or does it need to be dry docked?**
_____

Eyes: N □ A □, **Nares:** N □ A □, **Tympanum:** N □ A □, **Beak:** N □ A □, **Oral Cavity:** Glottis: N □ A □, tongue: N □ A □, Mucous membranes (color, ulcers, plaques): N □ A □
_____

Mark abnormalities on diagram on page 2.

Carapace: N □ A □, **Plastron:** N □ A □, **Ectoparasites/Epibiota (None=0, mild=1, mod=2, heavy=3): large barnacles , small barnacles , leeches, leech egg cases, skeleton shrimp, other (list species and load)**_____
_____
_____
_____

Skin: N □ A □, **Ectoparasites/Epibiota (list species and load):**
_____
_____
_____

Nails: N □ A □, **Hydration Status:** N □, <5% □, 5-10% □, > 10% □, **Body Condition Score** (1=emaciated, 2=underweight, 3=normal, 4= overweight, 5=obese): **_____**

Vent/Cloaca: N □ A □, **inguinal space palpation**: N □ A □

Musculoskeletal: Front flippers (joints, range of motion, fractures, wounds/abscesses, Nails) N □ A □; Hind flippers: N □ A □

Cardiovascular: N □ A □ Heart Rate (Doppler): **_____** bpm

Respiratory: N □ A □ Respiratory Rate: **_____** breaths per min

Diagnostic Procedures:

Radiographs: Yes □ No □

Interpretation:_____

_____

Blood work (COLLECT AS SOON AS POSSIBLE AFTER ADMISSION):
Collection site:_____

PCV: _____%, TS _____, glucose _____, WBC_____ %, _____,
Differential: heterphils_____, lymphs_____, azurophils_____, eosinophils_____,
Basophils_____, morphology_____

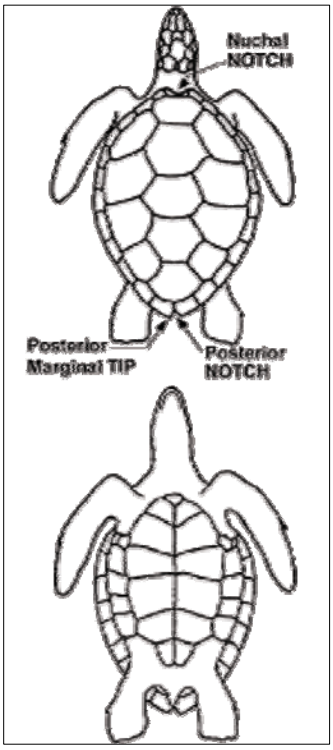
Samples for shipment to UF:
Blood smears (2 slides) □ Heparinized whole blood □ Plasma (3-6 mls) □

Blood culture: Yes □ No □ Results:

Fecal float/sedimentation: neg □ pos □, Direct: neg □ pos □

_____

Other diagnostics:
In house:
Sent to other laboratories (list lab and date sent):



Clinical notes:

_____
_____
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LIVE ANIMAL SAMPLE CHECK LIST

A. Blood samples (Please collect as soon as possible after stranding):

- □ One tube whole blood (in lithium heparin)
- □ 3-6 mls plasma (collected in lithium heparin and separated ASAP)
- □ Two unstained blood smears

B. Requested opportunistic samples:

- □ Fecal sample

- □ Urine sample

Please include stranding date _____ and date of collection _____.
Ship samples on ice packs as soon as possible after collection to:

Dr. Brian Stacy
University of Florida, CVM
2015 SW 16th Ave
Room VC-83
Gainesville, FL 32608
352-283-3370

APPENDIX II
NEUROLOGICAL EXAMINATION (ADAPTED FROM CHRISMAN ET AL, JAVMA, 211(8), 1997)

While turtle is in the water					
Observations (Check appropriate box)					
Mentation	Alert □	Depressed □	Demented □	Stuporous □	Comatose □
Compulsive circling	None □	Both directions □	Left □	Right □	
Head posture	Level □	Tilted Left □	Turned left □	Tilted Right □	Turned Right □
Head movement	Normal □	Decreased □	Absent □	Uncoord □	Tremors □
Body posture	Level □	Tilted Left □	Tilted Right □	Pelvic float □	
Comments:					

Indicate appropriate number; Key: 4-uncoordinated; 3-increased; 2-normal; 0-absent; NE-not evaluated						
Visual avoidance Left eye			Thoracic limbs		Pelvic limbs	
Visual avoidance Right eye		**Movement**	L	R	L	R
General activity		**Strength**				
Righting response						
Tail movement						
Comments:						

While turtle is out of the water in ventral recumbency (Check appropriate box)					
Activity while lifted	Increased □	Normal □	Reduced □	Absent □	
Mentation	Alert □	Depressed □	Demented □	Stuporous □	Comatose □
Head posture	Level □	Tilted Left □	Turned left □	Tilted Right □	Turned Right □
Head movement	Normal □	Decreased □	Absent □	Uncoordinated □	Tremors □
Comments:					

While turtle is out of the water in ventral recumbency (Check appropriate box)			
Indicate appropriate number Key: 4-uncoordinated; 3-increased; 2-normal; 1-decreased; 0-absent; NE-not evaluated; NA-not applicable			
Cranial nerves	L	R	**Comments:**
I – Olfaction			
II,VII - Menace			
II,III – Pupillary reflex			
III,IV,VI – Strabismus			
V – Jaw tone/strength			
V,VII – Palp reflex			
VIII – Vestibular nistag			
IX,X – Swallowing			
XII - Tongue			

While turtle is out of the water in dorsal recumbency						
Indicate appropriate number: Key: 4-uncoordinated; 3-increased; 2-normal; 1-decreased; 0-absent; NE-not evaluated; NA-not applicable						
	Thoracic limbs		Pelvic limbs			
	L	R	L	R		
Movement					**Cloaca & Tail**	
Strength					**Cloacal nociception**	
Tone					**Tail movement**	
Flexor reflex					**Tail nociception**	
Crossed extensor						
Clasp response						
Dermal nociception						
Periosteal nocicep.						
Sensation	L	R	L	R		
Neck						
Scute						
Comments:						
Location of lesion(s):						

APPENDIX III
PROTOCOL FOR NECROPSY SAMPLE COLLECTION DURING MARINE TURTLE UNUSUAL MORTALITY EVENTS (UME'S)

Included in this packet is a twelve page guideline for data and sample collection for animals that die in association with unusual mortality events. This guideline consists of a coversheet, checklist of recommended samples to be collected and stored frozen, checklist of histopathology samples, and gross necropsy reporting form. Attempts have been made to facilitate a thorough, time-efficient postmortem examination and to insure that data sets are complete and remain organized and interpretable for management during an event and retrospective evaluation. Components and rationale for each are as follows:

1. Necropsy coversheet – Objectives:
    a. Retain identification by STSSN number and cross reference with patient name, case number, or other means identification.
    b. Identify the facility/agency of origin, prosector(s), critical dates, basic biology data, and brief inventory of antemortem and portmortem materials in a checklist format.
    c. Not included are comprehensive biological measurements on the STSSN form – this form should be completely filled out in all cases.
2. Frozen sample collection
    a. Only performed for codes 2, 3, F
    b. Consists of two options:
        i. a **prioritized sample checklist*** that includes samples essential for biotoxin testing, but also inclusive of samples required for other toxin testing and infectious disease diagnostics
        ii. a **comprehensive sample checklist** collected under ideal necropsy conditions

    *The intent is to provide a more time efficient option if resources, assistance, and/or time are limited.
3. Checklist of histopathology samples (Code 2 only)
    a. Basic list of samples necessary for complete histopathological evaluation
4. Gross necropsy reporting form
    a. Modified from Stamper et al. 1996 to create a more concise reporting form
    b. Some items were excluded to minimize redundancy or omit data rarely collected during marine turtle necropsies and/or of minimal diagnostic utility.

Contact information for SE regional marine turtle response network & UME collaborators*

Dr. Terry Norton, Georgia Sea Turtle Center

Phone: 912-635-4070 Email: tnmynahvet@aol.com, tnorton@jekyllisland.com

Dr. Brian Stacy, University of Florida, College of Veterinary Medicine

Phone: 352-283-3370 Email: Bstacy@vetmed.ufl.edu

Dr. Allen Foley, State Coordinator - Florida

Phone: 904-591-1285 Email: Allen.Foley@MyFWC.com

Ms. Dubose Griffin, State Coordinator – South Carolina

Phone: 843-870-3667 Email: griffind@dnr.sc.gov

Mr. Mark Dodd, State Coordinator - Georgia

Phone: 912-280-6892 Email: mark_dodd@dnr.state.ga.us

*Please contact one of the above individuals if you would like to be added to this list

Shipping address for archival samples / data sheets

Attn: Dr. Brian Stacy

Marine Animal Disease Laboratory

University of Florida, College of Veterinary Medicine

2015 SW 16th Ave

Room VC-83

Gainesville, Florida 32608

352-283-3370

(Please contact Dr. Stacy prior to any shipment and only ship Monday through Wednesday)

Necropsy coversheet

1. STSSN #: _____ Facility ID reference: _____
   (Sea Turtle Stranding and Salvage Network STSSN number) (Patient name / other stranding number)

2. Agency/Facility: _____
   Person(s) conducting necropsy: _____

3. ☐ Found dead ☐ Found alive, date of death: _ _ / _ _ / _ _

4. Date of necropsy: _ _ / _ _ / _ _

5. Species (codes): ☐Loggerhead (CC) ☐Green (CM) ☐Leatherback (DC)
   ☐Hawksbill (EI) ☐Kemp's Ridley (LK) ☐Olive Ridley (LO) ☐Unknown (UN)

6. Estimated age class: ☐Hatchling ☐Post-hatchling ☐Juvenile ☐Adult ☐Other

7. Stranding condition: ☐Code 1 (Live) ☐Code 2 (Fresh carcass) ☐Code 3 (Moderately decomposed) ☐Code 4 (Advanced decomposition) ☐Code 5 (Skeleton or dried remains) ☐Code F (Frozen carcass)

   Gross necropsy: Codes 2,3,F
   Frozen sample collection: Codes 2,3,F
   Histopathology: Code 2 only

8. Available antemortem data/samples

- ☐ Physical examination
- ☐ Neurological examination
- ☐ Blood parameters (Biochemical / Complete Blood Cell count (CBC)
- ☐ Frozen plasma/serum ☐ Whole blood ☐ Feces
- ☐ Microbiology ☐ Radiology ☐ Photographs
- ☐ Other: _____
  _____

9. Case data/sample inventory

- ☐ Copy of completed STSSN form
- ☐ Frozen sample checklist (check one)
  - ☐ Prioritized tox sample set ☐ Comprehensive sample set
- ☐ Completed necropsy report form
- ☐ Histopathologic samples

Necropsy sample checklist – UME protocol

Codes 2, 3, & F	Code 2 only	Codes 2,3,F (Select one - Priority or Comprehensive)	
	10% NBF	❒ Frozen-Priority	❒ Frozen-Comprehensive
Fluids			
Plasma or serum (2-3 mls)		❒	❒
Bile (5-10 mls) (collect with needle/syringe after opening body cavity)		❒	❒
Urine (5-10 mls)		❒	❒
GI contents			
Stomach contents (collect wall if empty)		❒	❒
Intestine contents		❒	❒
Feces (from colon/cloaca)		❒	❒
Any hard parts, bone, shell		❒	❒
Tissues		**Plastic (P) only**	**Plastic & Foil (F)**
Skin	❒		
Eye	❒		
Conjunctiva	❒		
Tongue	❒		
Skeletal muscle (3 sites: neck and two limbs)	❒		
Trachea	❒		
Lungs (two sections each [different areas]: right and left lung)	❒	❒ P	❒ P
Thyroid	❒		
Thymus	❒		
Heart (one section from ventricle and each atria)	❒		❒ P ❒ F
Aorta	❒		
Liver (one section from each lobe minimum)	❒	❒ P (left lobe)	❒ P ❒ F
Gall bladder	❒		
Spleen	❒		❒ P
Pancreas	❒		
Esophagus	❒		
Stomach	❒	❒ P (if empty)	❒ P (if empty)
Intestine (one section each: proximal, mid, distal)	❒		
Colon	❒		
Kidney (one section each: right and left kidney)	❒	❒ P	❒ P ❒ F
Gonads & ducts (oviducts / epididymis, vas deferens)	❒		
Adrenal glands	❒		
Urinary Bladder	❒		
Brain	❒	❒ P	❒ P
Spinal cord	❒		
Axillary nerve	❒		
Pituitary gland	❒		
Bone marrow (2 sections: margin of carapace and long bone/pelvis)	❒		
Fat (near kidney left side)	❒	❒ P	❒ P ❒ F
Other lesions – list below (on Page 11)	❒	❒ P	❒ P
Parasites – list below (on Page 11)	❒ 70% Ethanol		

Frozen samples: All samples should be stored frozen in whatever facility is available. Ultralow storage (-80°C) is preferred. For tissues, collect at least 300 grams or a fist-sized section. Collect in ziplocks or whirlpaks.

Formalin-fixed samples: Collect all tissues in 10% neutral buffered formalin. Include normal and abnormal-appearing tissues, especially the margins of lesions if possible. All tissue sections should be less than 1.0 cm thick (except eyes) and fixed in a formalin:tissue ratio of 10:1.

Sea Turtle Necropsy Report

EXTERNAL EXAM

Ab=Abnormal NF=No Findings NE=Not Examined

Descriptions-include color, number, size, distribution, texture of lesions

Nutritional condition: ☐Good ☐Fair ☐Thin ☐Emaciated

Carapace / Plastron ☐Ab ☐NF ☐NE

Trauma

☐Propeller wound ☐Puncture wounds ☐Missing scutes ☐Bites ☐Tumors

Description/additional comments:

Epibiota ☐Ab ☐NF ☐NE

Distribution ☐Shell only ☐Generalized (Head & appendages)

Epibiota types ☐Sponges ☐Barnacle ☐Polychaetes
☐Goose barnacles ☐Leeches ☐Amphipod
☐Bryozoans ☐Other _____

Description/additional comments:

Integument (Skin) ☐Ab ☐NF ☐NE

Trauma

☐Sloughing ☐Necrosis ☐Net wounds ☐Fishing line/rope ☐Tumors
☐Propeller wounds ☐Other _____

Region: ☐Head ☐Neck ☐Front Flippers ☐Rear flippers ☐Tail

Description/additional comments:

Eyes ☐Ab ☐NF ☐NE

Location ☐Right ☐Left ☐Both

Description/additional comments:

MUSCULOSKELETAL SYSTEM

Skeleton and joints ☐Ab ☐NF ☐NE
Joint/synovial fluid: Color_____
Characteristics: ☐Blood tinged ☐Cloudy/flocculent material ☐Plaques
☐Other_____ Viscosity:_____
Fractures ☐No ☐Yes, where? _____
Dislocation ☐No ☐Yes, where? _____
Deformities ☐No ☐Yes, where? _____
Description/additional comments:

Musculature ☐Ab ☐NF ☐NE
Characteristics:
☐Abcesses ☐Clotted blood ☐Pale
☐Gelatinized ☐Necrosis ☐Parasites ☐Cysts
☐Other (Specify) _____
Description/additional comments:

Coelomic cavity ☐Ab ☐NF ☐NE
Fluid amount: _____ ml
Color:_____ Viscosity:_____
Characteristics:
☐Clear ☐Cloudy/flocculent material ☐Blood tinged ☐Hemorrhage
☐Blood clots ☐Adhesions ☐Plaques ☐Gritty material (hard)
Peritoneum:
Characteristics:
☐Tumors ☐Abcesses /granulomas ☐Congested
☐Hemorrhage ☐Clotted blood
Description/additional comments:

RESPIRATORY SYSTEM

Trachea/Bronchi ☐Ab ☐NF ☐NE
Abnormal Tissue: ☐Trachea ☐Bronchi
Characteristics:
Mucosa: ☐White ☐Vessels congested with blood
☐Hemorrhage ☐Ulcers
Trauma: ☐Punctures ☐Lacerations
Fluid: ☐Serous ☐Mucoid ☐Purulent
Fluid or foam:
Description/additional comments:

Lungs ☐Ab ☐NF ☐NE

Characteristics:
Lesion location: ☐Left ☐Right ☐Both
☐Cranial ☐Caudal ☐Dorsal
☐Ventral ☐Middle
Distribution: ☐Diffuse ☐Focal ☐Multifocal
Severity: ☐Mild ☐Moderate ☐Severe
Description/additional comments:

ENDOCRINE SYSTEM

Thyroid ☐Ab ☐NF ☐NE

Characteristics: ☐Normal ☐Enlarged ☐Atrophied ☐Friable
Description/additional comments:

Parathyroid(s) ☐Ab ☐NF ☐NE

If unable to find, save lining over left thymus
Description/additional comments:

Adrenal gland(s) ☐Ab ☐NF ☐NE

☐Normal ☐Enlarged ☐Atrophied
Description/additional comments:

CARDIOVASCULAR SYSTEM

Pericardial sac ☐Ab ☐NF ☐NE

Fluid amount: _____ ml
Color:_____ Viscosity:_____
Characteristics:
☐Clear ☐Cloudy/flocculent material ☐Blood tinged ☐Hemorrhage
☐Blood clots ☐Adhesions ☐Plaques ☐Caseous material
Description/additional comments:

Pulmonary arteries & Aorta ☐Ab ☐NF ☐NE

Characteristics:
☐Thrombi ☐Plaques ☐Ruptures
Description/additional comments:

Cardiac atria ☐Ab ☐NF ☐NE

☐Left ☐Right ☐Both
Characteristics:
☐Flaccid ☐Stiff ☐Thickened ☐Dilated
☐Hemorrhage ☐Pale areas ☐Parasites
Description/additional comments:

Cardiac ventricle ☐Ab ☐NF ☐NE

Characteristics:

☐Abscess/granulomas ☐Masses ☐Scars (fibrosis) ☐Friable

Description/additional comments:

HEMOLYMPHATIC SYSTEM

Spleen ☐Ab ☐NF ☐NE

Characteristics: ☐Enlarged ☐Atrophied ☐Friable

☐Abcesses/granulomas ☐Masses ☐Scars (fibrosis) ☐Friable

Description/additional comments:

Thymus ☐Ab ☐NF ☐NE

Characteristics**:** ☐Enlarged ☐Atrophied ☐Friable

Color:_____

Description/additional comments:

Pancreas ☐Ab ☐NF ☐NE

Characteristics:

☐Loss of lobulation ☐Necrotic ☐Edema ☐Inflamed

Color: _____

Description/additional comments:

HEPATOBILIARY SYSTEM

Liver ☐Ab ☐NF ☐NE

Characteristics: ☐Enlarged ☐Atrophied ☐Friable

Color:_____

Types of Lesions: ☐Abcesses/granulomas ☐Cysts ☐Masses
☐Congestion ☐Fibrosis ☐Necrosis

Severity of Lesions: ☐Slight ☐Mild ☐Moderate ☐Severe

Other: ☐Fatty (greasy) ☐Friable ☐Cirrhotic ☐Fractured

Description/additional comments:

Gall Bladder / Bile ducts ☐Ab ☐NF ☐NE

Bile: amount_____ml

Characteristics:

Color: serosa:_____mucosa:_____bile:_____

Types of Lesions: ☐Abcesses/granulomas ☐Cysts ☐Masses
☐Congestion ☐Fibrosis ☐Necrosis

Other: ☐Friable ☐Stones ☐Gritty material

Description/additional comments:

ALIMENTARY TRACT

Oral Cavity & Pharynx ☐Ab ☐NF ☐NE

Characteristics:

☐Ulcers ☐Fluid ☐Vomitus
☐Congestion ☐Parasites ☐Barnacles
☐Broken beak ☐Mandible ☐Maxilla
☐Foreign bodies _____

Description/additional comments:

Esophagus ☐Ab ☐NF ☐NE

Characteristics:

☐Dilated ☐Constricted ☐Perforated
☐Fluid filled ☐Foreign bodies_____(SAVE)
☐Other _____
Mucosa: ☐Congested ☐Hemorrhagic ☐Ulcers ☐Necrosis ☐Thickened
☐Film on surface of mucosa

Description/additional comments:

Stomach ☐Ab ☐NF ☐NE

Characteristics:

☐Clotted blood ☐Thickened ☐Ruptures/laceration
☐Volvulus (twist) ☐Erosions
Ulcers: ☐Mild ☐Moderate ☐Severe
☐Focal ☐Multifocal ☐Focally-extensive ☐Diffuse
Color: mucosa_____serosa:_____contents:_____
Contents: ☐Empty ☐Fluid ☐Dilated with gas
☐Mucus ☐Sand ☐Rocks
☐Other:_____ ☐Foreign bodies_____(SAVE)
Food: ☐Fish ☐Bivalves ☐Crustaceans
☐Cephalopods ☐Gastropods ☐Other_____
☐Undigested ☐Partially digested ☐Digested
Parasites: ☐Yes ☐No ☐<50 ☐>50

Description/additional comments:

Mesentery ☐Ab ☐NF ☐NE

Characteristics:

☐Hemorrhage ☐Clotted blood ☐Masses
☐Parasites (trematodes in mesenteric arteries)-SAVE_____Number

Description/additional comments:

Small Intestine ☐Ab ☐NF ☐NE

Characteristics: ☐Empty ☐Bile ☐Digesta
☐Other _____ ☐Foreign bodies_____(SAVE)
Color: mucosa_____serosa:_____contents:_____
☐Torsion/volvulus ☐Perforation ☐Masses ☐Abcesses
☐Constrictions ☐Diverticula ☐Ulcers (#)_____ Location_____
☐Pseudomembrane

Description/additional comments:

Colon ☐Ab ☐NF ☐NE

Characteristics:
☐Torsion/volvulus ☐Perforation ☐Masses ☐Abcesses
☐Constrictions ☐Empty ☐Feces
☐Fresh blood ☐Tarry ☐Other _____
☐Ulcers (#)_____ ☐Pseudomembrane
☐Parasites (SAVE): _____Number
Color: mucosa_____serosa:_____contents:_____

Description/additional comments:

Cloaca ☐Ab ☐NF ☐NE

☐Swollen ☐Prolapsed ☐Mucosal pseudomembrane
Feces color:_____

Glans penis ☐yes ☐no

Description/additional comments:

URINARY TRACT

Kidneys ☐Ab ☐NF ☐NE

☐Abcesses/granulomas ☐Parasites ☐Cysts
Lesion location: ☐Left ☐Right ☐Both
☐Dialated with urine ☐Masses ☐Calculi
☐Congestion ☐Necrosis (focal, multifocal, diffuse)

Description/additional comments:

Ureters ☐Ab ☐NF ☐NE

Description/additional comments:

Urethra ☐Ab ☐NF ☐NE

Description/additional comments:

Urinary bladder ☐Ab ☐NF ☐NE

Characteristics:

☐Empty ☐Dilated ☐Thickened ☐Tumors

Color: mucosa:_____urine:_____

Mucosa: ☐Hemorrhagic ☐Ulcerated

☐Masses ☐Plaques ☐Necrotic

Urine: amount _____ml Consistency:_____

☐Gritty material ☐Clear ☐Cloudy/flocculent material ☐Blood tinged

Description/additional comments:

REPRODUCTIVE

Gonads ☐Ab ☐NF ☐NE

Characteristics:

Sex: ☐Male ☐Female

Maturity: ☐Mature ☐Immature

☐Enlarged ☐Involuted ☐Masses ☐Follicles ☐Necrotic

Description/additional comments:

Oviduct ☐Ab ☐NF ☐NE

Characteristics:

☐Enlarged ☐Dilated with fluid ☐Hemorrhagic ☐Friable

☐Tumors ☐Masses ☐Mucus ☐Eggs

Description/additional comments:

NERVOUS SYSTEM

Dura mater and inside calvarium ☐Ab ☐NF ☐NE

Characteristics:

☐Hemorrhage ☐Clotted blood ☐Abcesses/Granulomas

Description/additional comments:

Central Nervous System ☐Ab ☐NF ☐NE

[Divide the brain in half (longitudinally), place half in formalin and freeze the other half]

Characteristics: (denote location of change in comments)

☐Distended red vessels ☐Abcesses/Granulomas ☐Clotted blood

☐Hemorrhage ☐Asymmetry ☐Edema ☐Black nodules

Lesions: ☐ Brain ☐ Spinal Cord _____(location) ☐ Pituitary ☐ Meninges

Description/additional comments:

Peripheral Nervous System ☐Ab ☐NF ☐NE
Other (s):_____
Characteristics:
☐Granulomas ☐Hemorrhage ☐Asymmetry ☐Edema
Description/additional comments:

Other:
Internal Auditory Meatus: ☐Ab ☐NF ☐NE

Other information or description continuation page:

Necropsy ancillary diagnostics:

Microbiology:

☐Cultures ☐Aerobic ☐Anaerobic ☐Fungal Site(s):_____

Submitted to:_____

Parasitological studies:

☐Fecal analysis Other:_____

Submitted to:_____

Molecular diagnostics:

Test(s):_____

Submitted to:_____

☐Electron microscopy

Other: _____

APPENDIX IV
STANDARDIZED TISSUE SAMPLES FOR BIOTOXINS (INCLUDED IN NECROPSY PROTOCOL)

- Routine analysis will screen for brevetoxin, saxitoxin, and domoic acid (with other potential HAB toxins screened as warranted).
- For confirmation and analytical support, samples will be forwarded (as necessary) from FWRI to the NOAA/NOS CCEHBR Analytical Response team in Charleston, S.C.
- Samples (Acute): plasma/serum, urine, feces, stomach &/ intestine contents
- Samples (Tissue): liver, lung, gall bladder/bile, brain, kidney. (For Neurological Loggerhead Complex also collect cerebral cortex and spinal cord.)
- Condition: fresh frozen (-80º C preferred, but conventional freezer is adequate)
- Other: For newly recognized toxins (like BMAA) samples from controls are needed.
- Collect up to 10g or 10mL if possible. Stomach contents should be as representative as possible, so a larger sample may be necessary. Minimum sample size is 2 g or 2 mL, but the number and type of analyses may be limited for smaller samples.
- Store each sample separately in a plastic bag (or vial for fluids).
- Label each sample with the turtle's Stranding Network ID number, species, tissue type, and collection date.

Ship samples to the following address:

Leanne Flewelling
Florida Fish and Wildlife conservation Commission, Fish and Wildlife Research Institute
100 8th Ave. SE, St. Petersburg, FL 33701
727-896-8626
Leanne.Flewelling@MyFWC.com
(Marine Algal Toxin Specialist)

Analytical capabilities:

1. General toxicity assays: mouse bioassay (FWRI), Na channel-directed cytotoxicity assay (NOAA).
2. Both screening assays (antibody and/or activity-based) and analytical methods (HPLC or LC-MS) for all major algal toxins: for brevetoxin, saxitoxin, domoic acid, ciguatoxin, okadaic acid. (FWRI and/or NOAA).
3. FWRI Contact: Leanne Flewelling, FWC/FWRI, 727-896-8626, Leanne.Flewelling@MyFWC.com
4. NOAA Contact: Frances Van Dolah, NOAA/NOS CCEHBR, (843) 762-8529, Fran.Vandolah@noaa.gov

APPENDIX V
SAMPLES TO COLLECT FOR TOXICOLOGY (NON-BIOTOXIN):

Non-biotoxin toxicological testing will be guided by ancillary data including environmental data, site/event observations, and clinical and necropsy findings and are not necessarily included in initial event response protocol. Samples collected for biotoxin testing will be suitable for most diagnostic testing. The following are broad categories of compounds of interest:

1) Cholinesterase (currently limited to loggerheads)
   Samples: plasma (0.5 mL required)
   Condition: pre-mortem only
   Other: Plasma from minimum of two control animals required for assay.
   Analysis: Assay
   Contact: Nancy Szabo, UF Analytical Toxicology Core, 352.392.2243 ext 5570; szabon@vetmed.ufl.edu

Brain ChE is possible, but tissue must be collected <1hr from death. Brain from control also required for assay.

2) Degradable Contaminants (Organophosphates, carbamates, most pharmaceutical products)
   Samples: liver and kidney (2-10 g tissue required per class of compound)
   plasma/serum (0.5-2 mL required per class of compound)
   Condition: Fresh frozen. If organophosphate poisoning is suspected, arrange for immediate analysis (OPs degrade even in frozen tissues/fluids). *Can analyze for stable terminal metabolites even after long-term sample storage, but findings will be nonspecific for other than compound class.
   Analysis: GC-MS and LC-MS (method determined by suspected toxin)
   Contact: Nancy Szabo, UF Analytical Toxicology Core, 352.392.2243 ext 5570; szabon@vetmed.ufl.edu

3)Persistent Contaminants (Chlorinated hydrocarbons, heavy metals, most industrial wastes and by-products)
   Samples: liver and kidney (2-5 g tissue required per class of compound)
   Other: Control material generally not needed. For persistent toxins (which do not degrade easily) decomposed tissues can also be analyzed.
   Analysis: GC-MS primarily
   Contact: Nancy Szabo, UF Analytical Toxicology Core, 352.392.2243 ext 5570; szabon@vetmed.ufl.edu

APPENDIX VI
SOP FOR COLLECTION OF WATER SAMPLES FOR MICROALGAL ABUNDANCE AND TOXIN ANALYSIS

Equipment:

Weighted bottle on a rope, beta or Niskin bottle, or a bucket on a rope

Clean bottles to store sample, 500 to 1,000 mL

Brown/amber bottles to store fixed sample, 125 mL

Unacidified Lugol's solution

Disposable 5 mL pipettes

Paper towels or newspaper

Thermometer

Labels
Permanent marker
Data sheets
Cooler
GPS unit (if available)

Methods:

1. Collect the water samples in a clean container (bottle, Niskin bottle or a bucket) that has been rinsed with seawater from the sample location to removes residue. The sample should be taken at approximately 0.5 meters below the water surface.

2. Shallow samples can be collected with any kind of bottle or bucket. Deep samples can be collected in a beta or Niskin bottle, or weighted bottle on a rope, "trapping" the water at the chosen depths.

3. Unpreserved sample: Carefully pour some of the water into the 500ml-1L sample bottle and then rinse. Dump out the rinse water. Completely fill the sample bottle; avoid excessive turbulence and bubble creation, which can break up certain cells. Put on the lid.

*If the unpreserved sample is for both microalgal identification and toxins **OR** if both preserved and unpreserved water samples will be shipped immediately, **do not freeze**. Wrap the sample bottle in wet towels or newspaper, place in a Styrofoam (or other) cooler and ship by overnight courier. The evaporation of water from the wet paper towels or newspaper keeps the sample from heating up.

4. To preserve water samples with unacidified Lugol's solution, add about 2 mL of stock unacidified Lugol's preservative to 125 mL of sample in an amber/brown container. Cap tightly and invert several times to mix. The mixture should be the color of light to medium tea.
(FWRI can provide amber bottles with Lugol's solution pre-measured inside the bottle. In this case, do NOT pre-rinse the bottle with sample water. Just fill it and mix.)

5. Preserved samples can be shipped at ambient temperature – make sure cap is tight and place bottles inside ziplock bags. Unpreserved samples can be shipped at ambient temperature if being shipped within 24-hrs of collection. Otherwise, freeze as soon as possible and ship overnight, frozen and on ice.

6. Be sure to label all sample bottles with collection date, specific location, depth and sampler's name. Information should be written on the side of the sample bottle, not on the lid. If possible, take GPS coordinates at each site, and record temperature, salinity, dissolved oxygen, tide stage and wind direction on a data sheet.

To prepare Lugol's solution:
Potassium iodide – 1g
Iodine – 0.5g
Dissolve in 3 mLs of distilled water, then dilute to 50 mL with distilled water.
Note: the preservative will stain clothing.

APPENDIX VII
COLLECTION PROTOCOL FOR ENVIRONMENTAL SAMPLING DURING MORTALITY EVENT

I. For immediate response before any sampling kits can be mobilized:

- Water (min. 500 mL) can be collected at the stranding site in any clean bottle or jar on hand and shipped to FWRI overnight at ambient temperature for algal species identification and toxin testing. If they cannot be shipped quickly, then freeze them (-80 preferred, but whatever is available), but will then only be useful for toxin testing.
  (Refer to Collection and Treatment of Water samples SOP.)
- Anything else that is found dead in the area (fish, inverts, etc.) – collect and freeze
- On request, sampling kits containing bottles for preserving samples can be provided to the responding agency.
- Water samples should be both fixed (in Lugol's –bottles with fixative already inside will be provided) and frozen (500mL-1L). Preserved samples (with Lugol's solution) should be shipped in a separate container from frozen water and tissue samples.
- Collect 1 L of water and ship immediately or freeze for toxin analysis. Collect 3 125-mL water samples (at stranding site and 100 m to either side of site).
  (refer to Collection and Treatment of Water samples SOP.)

I. Offshore collection protocols

- Sampling kits containing bottles for preserving samples and bottom sampler can be provided prior to departure.
- Water – Collect from just below the surface (with a pre-rinsed bottle or bucket) and from just above the bottom. Collect both fixed (in Lugol's –bottles with fixative already inside will be provided) and frozen (500mL-1L). Preserved samples should be shipped in a separate container from frozen water and tissue samples.
- ***(See Collection and Treatment of Water samples SOP.)***
- If possible, collect a sediment grab from the bottom. From the grab, retrieve the top 2-3 cm and store in glass jar or plastic bottle with some water. Keep sediment samples at ambient temperature. Do not preserve or freeze.
- Identify methods to collect potential prey items for toxin testing depending on nature of die-off. If likely to be benthic then identify if "ships of opportunity" are available for water and benthic type dredge samples. (Alternatively scope out network of charter boat captains, boat operations etc for hiring during events. Needs to be guided with aerial overflights).

APPENDIX VIII
COST PROJECTIONS FOR LIVE TURTLE CAPTURES BY TRAWLER DURING A MORTALITY EVENT

SCDNR boat cost ..... $2,700/ day

Georgia Bulldog costs: 'Running day' currently $750.00

Contact person: Lindsey Parker, UGA Marine Extension Service, 715 Bay St, Brunswick, GA 31520
912-264-7331(desk),912-264-7312(fax),912-617-7054(cell)

RECHARGE POLICY FOR
THE R/V GEORGIA BULLDOG
THE UNIVERSITY OF GEORGIA
MARINE EXTENSION SERVICE
BRUNSWICK, GA 31520

This recharge policy is for the use of the Research Vessel GEORGIA BULLDOG. All charges below include the captain, crew, fuel, food, and other vessel operating costs.

LOCAL/IN PORT

$ 1,500	Less than 10 hours/day
$ 1,800	10 – 12 hours/day
$ 2,000	12+ hours/day

OUT OF PORT (NON-LOCAL)1

$ 1,800	Less than 10 hours/day
$ 2,000	10 – 12 hours/day
$ 2,200	12+ hours/day

OTHER CHARGES

$ 1,000	Cruise mobilization2
$ 1,000	Cruise demobilization2
$ 750	Running day
$ 1,100	Weather day
$ 1,100	Standby day

APPENDIX IX
SAMPLE SHIPMENT PROTOCOL – UNIVERSITY OF FLORIDA REPOSITORY

The University of Florida, College of Veterinary Medicine will serve as a central repository of samples and data collected during sea turtle mortality events and other relevant materials as needed. Facilities, including ultralow freezer storage, for this purpose have been provided by the National Marine Fisheries Service. The shipping address is as follows:

To the attention of: Dr. Brian Stacy
University of Florida, College of Veterinary Medicine
2015 SW 16th Avenue
Room: VC-83
Gainesville, FL 32608
(352) 392-2212 ext. 5788

Please contact Dr. Stacy in advance of any shipment and only ship materials Monday through Thursday.

Preferred shipping conditions:

Sample labeling: All samples/materials should be clearly labeled with STSSN#

Frozen samples: Any samples that have been stored frozen, such as tissues and fluids for toxicological testing, should be shipped on dry ice whenever possible to prevent thawing. If dry ice is unavailable, contact Dr. Stacy for other possible options.

Samples in fixatives (formalin/ethanol): Any fixative should be decanted to avoid shipping hazardous or prohibited substances and the container should be tightly sealed to avoid drying. Formalinized tissues can be wrapped in paper towel moistened with formalin. The type of fixative (e.g. 70% ethanol, buffered formalin) should be clearly indicated in the sample so that it can be replenished upon arrival. *Please include a completed necropsy form/report with any formalinized tissues.*

Files / Paperwork: Any paper materials should have the **STSSN #** clearly indicated in addition to any other identifier. For medical records, a completed copy of physical and neurological examination forms (Appendices I and II) should be included.