Interim Report

on the

Bottlenose Dolphin (*Tursiops truncatus*) Unusual Mortality Event Along the Panhandle of Florida March-April 2004

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I. EXECUTIVE SUMMARY

This interim report outlines the initial findings and the ongoing analyses in the investigation of the bottlenose dolphin (*Tursiops truncatus*) mortality event that began in March 2004. One hundred and seven (107) bottlenose dolphins stranded dead along the Florida Panhandle between March 10 and April 13, 2004. Hundreds of dead fish and marine invertebrates were also discovered in the area. The National Marine Fisheries Service formally declared the dolphin deaths an "Unusual Mortality Event" (UME) after consulting with the Working Group on Marine Mammal Unusual Mortality Events. A multi-agency investigation was initiated and is being conducted by federal and state marine wildlife officials working in partnership with private research organizations and universities.

Analyses conducted to date found brevetoxins (see Appendix B), naturally occurring neurotoxins produced by *Karenia brevis*, the Florida red tide, at high levels in the stomach contents of all dolphins examined to date and at variable levels in the tissues of these animals. Metabolite concentrations in tissues of fish or dolphins are not yet completed. The concentrations of brevetoxins observed in the analyzed subsample of the stomach contents are greater than or equal to those observed in previous marine mammal mortality events associated with Florida red tides (*Karenia brevis* blooms) in the Gulf of Mexico. In most of the dolphins, the first chamber of the stomachs was gorged with large amounts of fish, some of which were partially whole and undigested indicating recent feeding. Fish (planktivorous, herbivorous, and omnivorous fish species) collected from St. Joseph Bay on March 18 tested positive for brevetoxins in stomach contents and in muscle, liver, and gill tissues whereas fish collected on March 28-31 also tested positive for brevetoxin but at much lower levels (except in sea trout). Satellite imagery of the northern Gulf of Mexico indicated elevated chlorophyll levels in the UME area March 9-11, 2004 but water samples collected March 11 and later in the area of the UME did not contain significant quantities of *Karenia brevis*, although low levels of brevetoxins were detected. Thus, the source of the brevetoxins involved in this event remains uncertain, but the presence of toxic fish and water suggests that there was an undetected bloom somewhere either in the bay itself or in waters in which the fish or dolphins were feeding. A similar dolphin UME occurred in 1999-2000 in the same area of Florida and was correlated with a *Karenia brevis* bloom.

In addition to brevetoxin, low levels of domoic acid (see Appendix C) were detected in the stomachs, urine and feces of some dolphins. Further, *Pseudo-nitzschia delicatissima*, a diatom that can produce domoic acid was present at low to moderate levels in water samples. However, the levels of domoic acid detected were 1-2 orders of magnitude lower than those found in domoic acid related marine mammal mortalities on the California coast. Therefore, its role in the current event would appear to be secondary to brevetoxin if, in fact, it is involved at all.

The evidence based on gross and histological findings does not indicate an infectious process and viral testing has ruled out morbillivirus, a virus known to cause high morbidity and mortality in dolphin populations. The lack of evidence of infectious disease, the wide age class spread of the mortalities, and the fact that most animals had recently fed (demonstrated by full stomachs) does implicate a toxin of some type as one of the causes of the event. The Investigative Team is continuing to look at all potential causes of the mortality event and will conduct further analyses of the brevetoxin metabolites, prey and stomach contents, and genetic identification of the Florida Panhandle population. Additional work includes quantification of the estimated dose of toxins received by the animals, examination of any predisposing factors, and searching for evidence of any other toxicants that might be in dolphin prey species. The fact that southwest Florida has significant blooms of *Karenia brevis* almost annually without unusual numbers of bottlenose dolphin mortalities makes this investigation even more perplexing. In order to fully understand what is happening in these dolphin mortalities, the investigation will continue to work with an interdisciplinary team of scientists using an ecosystem approach to understand the factors that contributed to this mortality event.

II. INTRODUCTION

Between March 10 and April 13, 2004, 107 bottlenose dolphins (*Tursiops truncatus*) stranded dead along the Florida Panhandle coastline from Escambia to Franklin counties. The majority of the animals (70%) stranded in St. Joseph Bay and surrounding areas of Gulf County (Figure 1). On March 12, approximately 26 dolphins had stranded within a 3-day period. The annual average of dolphin strandings along the Florida Panhandle is eight (8) animals per year, so the high number of dolphin strandings in March 2004 prompted the National Marine Fisheries Service (NMFS) to initiate consultation with the Working Group on Marine Mammal Unusual Mortality Event (Working Group). The Working Group reviewed the available stranding data as well as historical records from the area for comparison, and recommended that NMFS declare the die-off an official "Unusual Mortality Event" (UME).

A. Unusual Mortality Event (UME) Program and the Working Group on Marine Mammal Unusual Mortality Events

Title IV of the Marine Mammal Protection Act (MMPA) established the "Marine Mammal Health and Stranding Response Program" (16 U.S.C. 1421 *et seq.*), and section 404 of the MMPA specifically directs the Secretary of Commerce (through NMFS) to administer responses to UMEs. The MMPA characterizes UMEs as mass mortalities and/or strandings of marine mammals that are: (1) unexpected; (2) involve a significant die-off of any marine mammal population; and (3) demand an immediate response. Additional considerations include strandings of unusual species, locations, time of year, etc.

Section 404 of the MMPA also established the Working Group whose role is to: (1) determine whether or not a UME is occurring; (2) determine if/when a UME has ended; and (3) help develop a contingency plan for UME response. The Working Group is composed of 12 scientific experts with experience in marine mammal science, marine mammal veterinary science and husbandry, biomedical specialties, and/or marine conservation. There are seven criteria that the Working Group uses to determine if a marine mammal mortality event is "unusual" (see Appendix A). A single criterion or combination of criteria may indicate the occurrence of an unusual mortality event.

B. Historic UMEs in the Gulf of Mexico and Florida Panhandle

Since 1992, eight marine mammal unusual mortality events have been investigated in the Gulf of Mexico. Three of those involved manatees and were associated with *Karenia brevis* blooms and brevetoxins in tissues and gut contents. The remaining five UMEs involved bottlenose dolphins, and two of those occurred in the Florida Panhandle counties. In 1993-1994 an UME of bottlenose dolphins started in the Florida Panhandle and spread west with most of the mortalities occurring in Texas. That mortality event was caused by a morbillivirus epidemic. Between August 1999 and February 2000, the Florida Panhandle experienced a second UME of at least 120 bottlenose dolphins coincident with *Karenia brevis* blooms and fish kills. In both the 1999-2000 and 2004 UMEs other species of marine life such as fish, invertebrates and birds died. The current UME and the 1999-2000 UME both started in the same location (St. Joseph Bay) and extended along the seven counties of the Florida Panhandle (Escambia, Santa Rosa, Okaloosa, Walton, Bay, Gulf and Franklin Counties). The 1999-2000 UME persisted over a long period of time (9 months) and was spatially and temporally concurrent with a *K. brevis* bloom that moved from east to west. Brevetoxicosis was implicated as the cause of the 1999-2000 UME, but carcass condition limited the investigation and no definitive diagnosis was made.

C. Historical Knowledge of Bottlenose Dolphin Populations and Strandings Along the Florida Panhandle

The historical average of bottlenose dolphin strandings for the Panhandle counties over the last 10 years is eight (8) animals per year excluding years when a UME occurred. However, little is known of bottlenose dolphin behavior, ecology, and stock structure in the Florida Panhandle, including the region in and around St. Joseph Bay. To the best of our knowledge, no systematic research was performed prior to the 2004 UME to determine the daily or seasonal patterns of use of St. Joseph Bay by bottlenose dolphins, or their broader ranging patterns or genetic composition as these might relate to stock structure.

At other sites around the southeastern United States, a variety of techniques have been applied to determine stock structure, including among others: (1) biopsy darting to obtain small skin samples for genetic marking; (2) capture, sample, and release efforts to obtain genetic samples and apply tags or other markings; (3) photographic identification and re-sighting using distinctive natural markings on dolphin dorsal fins; and (4) radio-tagging and tracking. In the absence of specific empirical data identifying stocks of dolphins in the coastal waters of the panhandle, NMFS has used the approach of designating each of the 33 bays, sounds or estuaries in the US Gulf of Mexico as separate putative stocks, until such time as better data become available clarifying stock identification. The NMFS *U.S. Atlantic and Gulf of Mexico Marine Mammal Stock Assessments - 2000* (using 1993 survey data) reports that no dolphins were observed in St. Joseph Bay and surrounding waters during aerial surveys, so it was not designated as a putative stock.

The appearance of two die-offs of over 200 animals in the last five years, as well as the recent biopsy darting and photographic identification work with dolphins in St Joseph Bay and vicinity, indicate clearly that the 1993 aerial survey findings of no dolphins present in the Bay are out of date and do not provide sufficient information to determine impacts of these mortality events on coastal populations or stocks.

III. METHODS AND RESULTS

A. Dolphin Sampling

The dolphin strandings were initially clustered temporally (at least 30 dolphins within the first four days from March 10-13) and spatially along the Florida Panhandle, with the majority of strandings (70%) in St. Joseph Bay (Figure 1). The first three dolphins were reported on March 10 and the last two dolphins were reported on April 13. All of the 107 dolphins involved in the UME were discovered dead and no live strandings were reported. A variety of samples were collected and analyzed by several collaborators (Table 1).

The demographics of the strandings indicate that both sexes and most age classes were involved. The sex distribution of the stranded animals was approximately equal: 36 females and 46 males, with 25 carcasses of unknown sex. Age estimates based on growth layer groups of teeth have not been completed, therefore for this report age estimations are based on length to age curves for bottlenose dolphins in the Gulf of Mexico (Fernandez and Hohn, 1993; Read et al., 1993). Carcass measurements of 100 animals (not including two fetuses) show that length ranged from 76 cm (premature parturition) to 280 cm (adult) (Figure 2). Comparing the results of length classes with historic stranding records for this region (n=51, over 7 years), it is clear that a higher number of animals over 210 cm are represented in this die-off.

Gross Necropsy Findings:

Of the 107 dolphins involved in the UME, gross necropsies were conducted on 46 animals, some samples (e.g., teeth and skin) were collected from 44 animals, and the remaining animals (n=17) were located and recorded but not sampled due to logistical constraints and/or carcass condition. Blubber, liver and kidney were collected from four mildly decomposed animals for the National Marine Mammal Tissue Bank.

Carcass decomposition was classified into five categories: Code 2 - fresh (19 animals); Code 3 - mildly decomposed (39 animals); Code 4 - moderately decomposed (46 animals); Code 5 - severely decomposed (2 animals); and unknown (1 animal). No live stranded animals (Code 1) were found during the event. Most of the fresh animals were detected in the first half of the event, and the last known Code 2 animal was recovered on April 1.

Most animals were found to be in good nutritional condition, however a few animals were considered to be thin for this season as compared to winter season dolphin ranges in

Sarasota, Florida. Studies have shown that blubber thickness is affected by season in bottlenose dolphins in the Gulf of Mexico. Blubber thicknesses ranged from a low of 0.8 cm in a 268 cm male to a high of 2.3 cm in a 205 cm female using the mid-lateral measurement position from 19 animals. Gross findings varied with each animal, but pulmonary changes suggestive of pneumonia were noted in a number of animals which was further characterized histologically. Some animals possessed mild to moderate parasite infestations which was characterized by individuals with pterygoid sinus trematodiasis, pulmonary nematodiasis, gastric trematodiasis and enteric cestodiasis. There was evidence in a few animals of sediment in the trachea and primary and secondary airways, a characteristic that is seen in some animals that strand live but die on the beach. Many animals had evidence of generalized lymphoid stimulation, which was characterized by multiple enlarged lymph nodes. A number of animals had empty urinary bladders or contained a scant amount of opaque, pasty material with few containing collectable urine for analysis (10 urine samples collected). The majority of animals demonstrated a distended stomach with partially digested contents. Many animals had intestines which contained scant ingesta (n=11).

Stomach Content Analyses:

Stomach contents from 28 of 50 dolphins have been analyzed to date through the identification of fish remains and hard parts. Gastric subsamples were collected for biotoxin analyses from all 28 dolphins. Semi-digested fish found in the dolphin stomachs were collected for toxin analyses: menhaden from 16 dolphins; a puffer fish from 1 dolphin; and spot, silver perch, kingfish, pinfish and others from 11 dolphins. Two preliminary findings stand out:

(1) Dolphins appear to have gorged on food prior to death, as most stomachs were packed to capacity. Mean stomach content values were 773 g, with 10 samples weighing > 1 kg (maximum: 2.258 kg). These figures are significantly higher than those obtained from the 2001 bottlenose dolphin UME that occurred in the Indian River Lagoon (IRL) system on the east coast of Florida. During the 2001 IRL event, dolphins were generally emaciated and had very little food matter in their stomachs (mean of 36 g, n= 14) comprised mostly of fleshless fish bones. In addition, several of the IRL animals had empty stomachs. Compared with other studies in the southeastern U.S. (Figure 3), the 2004 UME dolphins from the Florida Panhandle stranded with considerably fuller stomachs than stranded dolphins in other areas. Data obtained from stranded dolphins in Texas (1987-1988) also showed high stomach mass values, but dolphins in that area are believed to gorge on fish discards from shrimp boats.

(2) Menhaden was the dominant prey species in at least 14 of the 28 (50%) dolphins examined, judging by the large number (dozens) of semi-digested fish found in each stomach. A sub-set (n= 19) of the undigested menhaden ranged from 9.5 to 16 cm, with most in the 14-15 cm size class. Sciaenid fish (e.g., silver perch, spot, kingfish) were dominant in 11 (39%) of the other samples. A few dolphins had undigested shrimp remains in their stomachs, and others had hundreds (possibly thousands) of fish ear bones (otoliths) of very small fish, mostly

croakers of the family Sciaenidae. These findings are similar to those found for Texas dolphins, where feeding in association with shrimpers is known to occur.

Virology:

Tissues obtained from 26 dolphins have been analyzed to date to determine pathogen presence using polymerase chain reaction (PCR) and reverse transcription PCR (RT-PCR). To date, all samples have been negative for morbillivirus (50 samples from 24 animals), influenza A virus (14 samples from 8 animals), and influenza B viruses (14 samples from 10 animals) as well as calicivirus (26 samples from 15 animals), cetacean poxvirus (3 samples from 3 animals), herpes virus (10 samples), and parapoxvirus (3 samples from 3 animals).

Histopathology:

Histopathological examinations were performed on partial to complete necropsy submissions from 38 dolphins (14 females and 24 males). No cause of death was evident histologically, and there was no histological evidence of infectious disease. Autolysis and postmortem bacterial overgrowth ranged from moderate to advanced, occasionally limiting the histological evaluations.

The vast majority of the dolphins appeared to be in good nutritional condition with mild to moderate fat atrophy only present in 6 of the 38 animals (16%) examined. Common histological lesions included prominent eosinophilia within multiple tissues (34/38), parasitic pneumonia (28/38), angiomatosis (20/38), mild myocardial fibrosis (11/38) and mild hepatic fibrosis (9/38). Less common lesions included mild perivascular brain edema (4/38), mild chronic interstitial nephritis (4/38), lymphocytic laryngitis (2/38), lymphoid hyperplasia (2/38), mild cerebral gliosis (1/38), mild lymphocytic meningitis (1/38), multifocal chronic myocarditis (1/38), and a pleural adhesion (1/38).

The prominent eosinophilic inflammation within multiple tissues is most likely associated with parasitism, a common finding in wild marine mammals. The changes within the liver are suggestive of a previous parasitic infection. Angiomatosis, a common proliferative lesion that occurs in the lungs and lymph nodes of dolphins, is thought to be clinically insignificant. Mild myocardial fibrosis is a fairly common finding in adult dolphins, and is usually clinically insignificant. The mild perivascular edema seen in a few dolphins is a common, nonspecific finding in the brains of stranded dolphins. Lymphoid hyperplasia is a nonspecific finding in response to antigenic stimulation. The significance of the myocarditis is uncertain since the inflammation was not associated with degenerative changes within the myocytes. The causes of the remaining lesions were not evident. They were all mild, and not likely associated with the death of the dolphins. Gross and histological lesions of brevetoxicosis are not well established or are nonspecific except in the case in inhalation brevetoxicosis. Gross lesions from the manatees with inhalation brevetoxicosis included nasopharyngeal and pulmonary edema and hemorrhage. Histological lesions in manatees with inhalation exposure included the above plus catarrhal rhinitis. No such gross or histological lesions were seen in the current investigation.

Organochlorines:

Concentrations of persistent organic pollutants ("POPs", *i.e.*, polychlorinated biphenyl (PCB) congeners, DDT and its derivatives, and other organochlorine insecticides) in bottlenose dolphin blubber samples collected from these animals were determined and compared to those observed in free-ranging Sarasota Bay dolphins captured during health assessment work. Only juvenile animals were used in this comparison because it has been shown that POP concentrations in juveniles are not statistically different between genders and juvenile animals can be identified by their length. The use of juveniles was also an attempt to control for age since that can influence POP levels in cetaceans.

Eighty PCB congeners, DDT and metabolites (DDE and DDD), oxychlordane, dieldrin and mirex were determined in full depth blubber biopsies from 23 juvenile dolphins collected from Sarasota Bay (collection interval: 2000 to 2004) and seven full depth blubber biopsy samples collected from Code 2 dolphins that stranded during the UME event. Samples were analyzed by gas chromatography with mass spectrometry at the NIST Charleston Laboratory. Total PCBs (sum of all measurable congeners) and total DDT (sum of 2,4'- and 4,4'-DDD, -DDE, and DDT) were not statistically different between the UME and Sarasota animals. The geometric mean of total PCBs was 18.5 mg/kg wet wt. (range 6.4 to 52.5 mg/kg wet wt.) and 16.5 mg/kg (range 12.3 to 24.7 mg/kg wet wt.) for the Sarasota and UME animals, respectively. The geometric mean of total DDT was 9.7 mg/kg (range 3.7 to 25.9 mg/kg wet wt.) and 9.6 mg/kg (range 6.3to 21.8 mg/kg wet wt.) for the Sarasota and UME animals, respectively. Based on the scientific literature, these levels are above those thought to have toxicological significance in delphinids (e.g., immunosuppression). Other organochlorine pesticides tended to be much lower in the UME animals than in the Sarasota animals. For instance, concentration (geometric mean) of dieldrin in blubber was 0.71 mg/kg (range 0.26 to 2.3 mg/kg wet wt.) in the Sarasota animals versus 0.097 mg/kg the UME animals (range 0.065 to 0.13 mg/g wet wt). Lipid content in blubber was not significantly different between the Sarasota dolphins and the six juvenile males from the UME analyzed to date, suggesting that these 6 animals were not thin at the time of their deaths.

Biotoxins:

Tissue and fluid samples from 38 dolphins along the Florida Panhandle (Figure 4) were analyzed for brevetoxins using multiple methods (competitive ELISA, Radioimmunoassay, Receptor Binding Assay, LC/MS, and reversed phase chromatography coupled to tandem mass spectrometry). All dolphins tested had high levels of brevetoxins in their stomach contents and/or feces (Figure 5). Significantly lower concentrations were present in tissues. The pattern of metabolites in tissues has not been completed but will provide insights into metabolism and potential toxicity. Brevetoxins were also measurable in the urine of most of the few animals tested (n=10). Brevetoxin concentrations varied widely between individual animals and between tissues but had a narrower range of values in 2004 than observed in the 1999-2000 dolphin UME along the Florida Panhandle. In addition, the levels found in stomachs of the 2004 UME dolphins were greater than those measured in the 1996 and 2003 manatee mortalities on the west coast of Florida (Figure 6). Although the toxic effect level of brevetoxin and its metabolites remains poorly defined, its presence at high levels in all animals tested supports brevetoxicosis as a differential diagnosis for this mortality event.

Trace levels of domoic acid (using receptor binding assay, ELISA and LC tandem mass spectroscopy) were found in the stomach contents and feces of some, but not all, of the stranded dolphins tested. *Pseudo-nitzschia* is not uncommon in the waters along the Florida Panhandle coast and, as there is no data on the natural levels of domoic acid in the food web from this part of the Gulf of Mexico, it is unclear if the trace amounts of domoic acid in the stomach contents and feces of these animals are significant to this mortality event.

Genetic Analyses:

Genetic analyses were performed on 64 sampled dolphins to determine whether the UME animals belonged to coastal or offshore stocks of bottlenose dolphins using DNA sequencing of a portion of the mitochondrial DNA genome (methods described in Rosel et al. 1999). Results indicate that all 64 animals were of the coastal morphotype.

B. Environmental Sampling

Satellite Imagery:

Satellite ocean color imagery has been used effectively for over four years as part of a monitoring program for *Karenia brevis* to support state and federal activities. The primary sensor is on the Sea-viewing Wide Field-of View Sensor (SeaWiFS). Data and analyses are typically provided to the state as a monitoring and forecasting tool for the blooms. These analyses have aided with other blooms, such as *Pseudo-nitzschia*, but are most effective with *Karenia* blooms. Detection is limited to about 50,000 cells L^{-1} of *Karenia*, which is much higher than that required for shellfish closures, but about the minimum at which human respiratory impacts occur. Images of total chlorophyll concentration and images showing the presence of new blooms are normally used as part of the analyses (Figures 7-12).

On February 28, a chlorophyll feature (2-4 μg/L) occurred about 12 nautical miles South-Southwest of Cedar Key (Figures 8-9). In January 2004, a bloom of *Karenia* appeared near Clearwater Florida. Analyses of imagery and winds indicates that the bloom originated south of Cedar Key, making it possible that a *K. brevis* bloom was present in the Cedar Key area. This region is problematic, with many frequent blooms.

On March 2, an area was flagged for new blooms from Cape San Blas to near Tyndall Air Force Base. Extreme elevated chlorophyll and a new bloom was evident in St Joseph Bay on March 9-11 (Figure 10). This characteristic of a bloom only in the Bay was observed in 1999 when a bloom of *K. brevis* occurred in St. Joseph Bay in August. Cell counts from water samples collected on March 11 contained *Pseudo-nitzschia* but not *Karenia brevis* (see HAB Monitoring), so it was assumed that the elevated chlorophyll was the result of a *Pseudo-nitzschia* bloom. The presence of brevetoxins in water at low levels (March 12, see Toxicity Testing) and fish from St. Joseph Bay suggest that

K. brevis may also have been present but was gone by the time samples were collected for phytoplankton counts. By March 18, slightly elevated chlorophyll levels were observed from the east end of St. George Island, offshore St. Joseph Bay and from Cape San Blas to 30 N.

Between April 12-15, a front passed through the UME area. Strong southerly winds followed by strong North-Northwesterlies caused mixing in the St. Joseph Bay area and resuspension of benthic material. This resulted in a rapid increase in chlorophyll that persisted for a few days and then diminished (Figures 11-12). Between April 16-18, two bloom patches were observed offshore and fish kills that were reported in St. Joseph Bay and 20 miles to the south of Cape San Blas.

Harmful Algal Bloom (HAB) Monitoring and Toxin Analyses:

<u>HAB monitoring</u>

Two dedicated cruises, one of which was funded by the CSCOR HAB Event Response Program and one cruise of opportunity, were conducted and water samples were collected at over 115 stations (Figure 13) from March 11 to May 5, 2004. Microscopic (both light and electron microscopy) identification and enumeration of phytoplankton and toxin analyses were conducted on the samples. During the event, as often as was feasible, water samples were collected in areas flagged by NOAA satellite imagery as potential HAB events.

No significant populations of *Karenia brevis*, were observed in 233 counts of samples from St. Joseph Bay and the surrounding offshore region in surface waters or at depth. *Pseudo-nitzschia delicatissima* was observed at low concentrations (0 to 1.7×10^4 cells L^{-1}) outside St. Joseph Bay, and at moderate concentrations (0 to 4.6×10^5 cells L^{-1}) inside the Bay. All stations measured had dissolved oxygen concentrations >7.2 mg L^{-1}, ruling out the involvement of low oxygen concentrations in the event.

<u>Toxin Analyses</u>

Brevetoxins: 84 water samples and 60 fish (both live-caught and from fish kills, total of 128 samples) were analyzed for brevetoxin by competitive ELISA. All water samples collected outside of St. Joseph Bay were negative, while those collected inside the bay contained low levels of brevetoxin (0.72 – 1.1 μg/L on March 12) in the absence of any apparent *Karenia brevis* cells.

Several species of fish were collected live and from fish kills in St. Joseph Bay. Most contained elevated levels of brevetoxin. Fish tissues examined included gill (64-798 ng/g), liver (865-4,655 ng/g), stomach contents (51-9,000 ng/g) and muscle (nd-473 ng/g). Fish collected live in St. Josephs Bay on March 18 contained variable levels of brevetoxin in their stomach contents. Burrfish (carnivores), spot (omnivores), and pinfish (herbivores) had high levels of toxin in their stomach contents (>1000 ng/g), whereas Gulf flounder and sardine collected at the same time were negative. Whether this reflects feeding habits of these species or individual variation is uncertain based on the small number of samples analyzed to date. Live fish collected on March 28 and 31 (n=47) included Spanish mackerel, pinfish, flounder, southern codling, spot, sea trout, bluefish, and thread herring. Brevetoxins were measurable but below 1000 ng/g in the stomach contents of all fish except for the sea trout, which contained 4,000 ng/g. In addition brevetoxins were found in the flesh of some fish.

Domoic Acid: Eighteen water samples were analyzed for domoic acid by ELISA. Although *Pseudo-nitzschia* was present and identified as *P. delicatissima*, all water samples were below the level of detection of the ELISA kit for domoic acid.

Mortality Reports in Addition to Dolphins

Fish kills and other animal mortalities were reported in St. Joseph Bay as follows:

03/11/04	Limited number of dead fish and horseshoe crabs were reported
03/14/04	Report of many dozens of lightning whelk at the Bay entrance (may have been empty shells)
03/18/04	Hundreds of dead horseshoe crabs; hundreds of dead jellyfish, one dead cormorant; dead red drum; numerous baitfish were swimming in circles at the surface; one sick laughing gull and cormorant exhibited neurologic signs
03/23/04	Fish kill involving spot, bay anchovy, cowfish, and striped burrfish. Additionally horseshoe crabs
04/02/04	Red drum, hundreds of dead adult horseshoe crabs, two dead pelicans, one egret, and one loon
04/12/04	Red drum
04/19/04	Red drum
05/06/04	From Pig Island, SW corner of the Bay - red drum, Gulf killifish, spotted sea trout

20 miles south of Cape San Blas:

04/14/04	Red drum and thread herring

These reports were not necessarily verified in every case, nor are they necessarily separate events, nor were necropsies conducted on all animals. Biotoxin analyses have not been completed for all the samples. General field observations and behavioral symptoms of moribund or sick animals are typical of exposure to *Karenia brevis* blooms.

Shellfish Monitoring for Harmful Algal Bloom Species

The only shellfish harvesting area in the immediate vicinity of the UME is the St. Joseph Bay Shellfish Harvesting Area. The area was temporarily closed at sunset on November 19, 2003 due to the detection of *K. brevis*. Therefore, no commercial or recreational harvesting of shellfish was allowed following November 19, 2003 including the time that the UME occurred. Waters surrounding the St. Joseph Bay Shellfish Harvesting Area are unclassified and commercial and recreational shellfish harvesting is never allowed.

Results from samples of shellfish meats for brevetoxins continued to be above the established regulatory level until April 29, 2004 (even though *Karenia brevis* cells were not present). The area was reopened to harvest at sunrise, on April 30, 2004 due to nontoxic bioassays for brevetoxin. Results from shellfish samples collected from the area showed that domoic acid concentrations were well below the established regulatory limit for public health protection. Therefore, recognized shellfish public health concerns were and continue to be adequately and appropriately addressed.

IV. DISCUSSION AND FUTURE PLANS

A. Comparison with Other UMEs

In the last decade there have been several UMEs of marine mammals that have been attributed to or correlated with harmful algal blooms. The most common harmful algal bloom in the Gulf of Mexico is caused by the red tide organism *Karenia brevis*, known to cause Neurotoxic Shellfish Poisoning and respiratory irritation in humans. The biotoxin brevetoxin and its metabolites produced by *Karenia brevis* were implicated in manatee mortality events in Florida in 1963, 1982, 1996, 2002, and 2003. Also, brevetoxin and its metabolites were suspected as a possible cause for bottlenose dolphin die-off events in Mississippi during 1996 and along the Florida Panhandle during 1999-2000. Although *Pseudo-nitzschia* spp. blooms are known in the Gulf of Mexico, no marine mammal mortality events have been attributed to these blooms or domoic acid (the related biotoxin) in this region. We must note that this is the first time that domoic acid has been analyzed in tissues from marine mammals that have stranded in the Gulf of Mexico, therefore no baselines exist for this biotoxin in marine mammals in the Gulf of Mexico. However, *Pseudo-nitzschia* blooms and subsequent domoic acid intoxication have been associated with sea lion and dolphin mortalities in California (1998 to 2003) and humpback whale mortalities on George's Bank (2003). The concentrations of domoic acid found in the bottlenose dolphin samples that were collected during this event were much lower than those found associated with previously documented marine animal mortalities. Therefore domoic acid intoxication is not considered as a primary cause of the 2004 dolphin UME, but has not been ruled out as a contributing factor.

B. Insights into Dolphin Behavior and Feeding in the Florida Panhandle

During Chicago Zoological Society/Florida State University biopsy sampling in April-May 2004, dolphins were frequently observed feeding on fish discarded from shrimp trawlers, while anchored or drifting and culling fish in St. Joseph Bay. Active trawlers were not observed in the bay -- it appeared that they trawled elsewhere and culled their catch in the sheltered waters of the bay prior to offloading their product at Port St. Joe. Croakers (Sciaenidae) are the predominant finfish by-catch group in the Gulf of Mexico shrimp fishery, which targets organisms (e.g., shrimp, bottom fish) living on or near the bottom. It has been suggested that the fact that some dolphins in St. Joseph Bay obtain at least part of their daily diet from the fish caught outside of the bay by shrimp trawlers and provided to the dolphins in the bay may have potential implications for evaluating the source of toxins in the stomach contents of dolphins dying during the UME.

Menhaden, on the other hand, are caught in large numbers in purse-seine nets. The biopsy darting team also observed purse-seine vessels returning to Port St. Joe, but the boats were neither fishing nor culling in the bay at the time they were observed.

C. Continuing Studies:

Acoustic/Blast Trauma -- Local citizens reported that the Department of Defense was conducting military exercises off the coast of the Florida Panhandle in March 2004. Based on the gross examinations, there was no physical evidence of blast or acoustic trauma in the 22 freshest dolphin carcasses. However, heads and ears collected from a subset of these animals will be examined specifically for blast and acoustic trauma using CT scanning methodology and histopathology. NMFS is working with the US Navy to determine what types of activities were conducted, and when and where they occurred, to compare with stranding data.

Life History – Demographics and life history parameters are essential components of UME investigations and provide tremendous insights into the real epidemiology of the event. Life history factors that will be critical to this investigation include such factors as: age determination, prey or feeding strategies (what do the animals eat?), and population or stock identification and assessment (who are the animals and where do they go). Stable isotope analyses will be conducted to determine historic feeding habits.
Age Determination -- Age estimations of the dolphins involved in the UME will be obtained by analyzing growth layer groups of teeth and reproductive organs. Reproductive status will also be determined.
Feeding (Stomach Content Analyses) -- Detailed dietary studies will be completed for all available UME samples (n= 50), including the contribution by biomass of the most important prey species. In addition, samples obtained from the 1999-2000 UME (n= 70 to 80) will be analyzed for comparative purposes.
Genetics -- Subsequent to the die-off, genetic samples from 30 live bottlenose dolphins in St. Joseph Bay and vicinity were sampled via biopsy darting. These samples will be compared to the UME animals using mitochondrial DNA sequencing and microsatellite analyses to help determine the origins of the dead dolphins. In addition, samples from the 1999-2000 event will also be analyzed and compared to the current mortalities.

Trophic transfer and metabolism -- There is increasing evidence that animals can be exposed to brevetoxins by multiple routes (inhalation and ingestion), though different vectors (prey, feeding habits, or directly consuming the harmful algae), and under different time scales. The mechanisms and timing of movement of biotoxins through the ecosystem and food web are critical to understanding risks of exposure and risks to populations. In addition to examining prey types and biotoxin levels in prey, metabolism of brevetoxins through the various levels of the food web will also be examined.

Biotoxin Dosage Determination -- Additional work will be conducted to quantify the estimated dose of toxins received by the animals and search for evidence of any other toxicants that might be in dolphin prey species and to compare the results with previous

UMEs. The toxicity of multiple biotoxin exposures in marine organisms using animal models will be useful to evaluate as part of long range studies.

D. Future Studies

This section outlines specific studies that need to be conducted to better understand this UME , the role of brevetoxins in UMEs, and the impacts of repeat UMEs in this geographic area. Although needed, there is no identified funding specific for these investigations.

Stock Structure -- To date the stock structure of coastal and estuarine bottlenose dolphins in the northern Gulf of Mexico has not been rigorously investigated using genetic and photo identification techniques. This should be a priority. Since so much is unknown about stock structure in this area, it will take some time to understand the impacts of these mortality events on a specific population(s) and risks from an increasing frequency of harmful algal blooms.

Health Assessments -- Health assessment indices should be developed and monitored through the use of live captures, sampling and release exercises in specific areas where anomalous mortality events have occurred (i.e., in St. Joseph Bay). Studies through live capture will be critical in determining risk factors in populations and which populations may be at risk. These studies will be essential in understanding any contributing or predisposing factors in the population that puts this population more susceptible to biotoxins, infections or other morbidity or mortality factors. This also includes assessment of strandings that occur to obtain more baseline information on mortalities and life history in this area.

Feeding Ecology -- Understanding the trophic ecology of wild dolphins is crucial in the monitoring of UMEs in which feeding is suspected to play an important role, particularly in areas where algal blooms are known to occur. A recommendation is made that samples (dolphin stomachs) be collected from stranded animals, so that baseline data on feeding and biotoxin concentrations can be obtained for the Florida Panhandle area.

E. Evaluation of UME Response

What went right?

- Long-term stranding response data were available and identified the unusual level of mortality almost immediately
- The response by the stranding network and colleagues was more organized and complete than during the 1999-2000 event
- Multiple federal and state agencies and outside partners worked cooperatively on a comprehensive investigation
- Appropriate expertise for various analyses had been previously identified, resulting in efficient dissemination of samples for various analytical tests

What challenges were faced?

- Current efforts to monitor blooms are guided primarily by the need to protect humans from Neurotoxic Shellfish Poisoning, not to protect marine mammals from Neurotoxin Fish Poisoning. Understanding the links between marine mammal mortality events in this area and the occurrence of HABs is not adequate to provide guidance for future monitoring to predict marine mammal events. Resources and equipment for the immediate response were limited
- Baseline information on the health, distribution, demographics, and abundance of dolphins in the area of the UME was limited, making interpretation of results more uncertain and tentative
- Baseline information with regard to concentrations of biotoxins (brevetoxin, domoic acid, and combinations of the two) in plankton, food items, digestive tract contents, body fluids, and tissues consistent with morbidity and mortality in bottlenose dolphins was, for the most part, limited or non-existent
- Baseline information on the levels of man-made environmental contaminants in this dolphin population and information on how these may interact with natural toxins was not available
- Currently we continue to be limited in biotoxin detection and quantification (for metabolites) by the state of the science and the limitations on standards and techniques
- Training and maintaining professional teams to respond to increasingly more complex investigations anywhere in U.S. waters is difficult and requires substantial resources

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VII. TABLES AND FIGURES

Table 1. Samples collected for analysis during the 2004 Florida Panhandle Unusual Mortality Event

Analyses	Purpose	Investigator/Laboratory	No. of Animals Sampled	Status
Gross Examination and Necropsy	Identification of gross causes of mortality and tissue sample collection	Various field investigators	Level 3* - 46 Level 2 – 44 Level 1 – 9 No Exam - 8	107 Completed
Acoustic trauma	To determine morphology and pathology of acoustic tissues	Woods Hole Oceanographic Institute	8	8 Pending
Aging and Life History	Estimate age and reproductive status	NOAA Fisheries, Southeast Fisheries Science Center, Beaufort, NC	51	51 Pending
Bacteriology	Bacterial identification to examine infectious processes and cause of death	Purdue University	2	2 Pending
		Hubbs Sea World Orlando	2	2 Pending
Brevetoxin/ Domoic acid	Tissue examination to identify and quantify biotoxins	Florida Marine Research Institute Florida Fish and Wildlife Conservation Commission	11	11 Completed
		Marine Biotoxin Program NOAA/NOS Charleston, SC	54	21 Completed 33 Pending
Genetics	DNA analysis to determine stock identity	NOAA/NOAA Fisheries Southeast Fisheries Science Center Baton Rouge, LA	65	65 Completed
Histopathology	Tissue examination to identify disease processes	Armed Forces Institute of Pathology	38	38 Completed
Immunoperoxidase	Examination to identify presence and distribution of brevetoxin in tissue samples	Harbor Branch Oceanographic Institute	TBD	Pending
Stomach Contents (Fish Species ID)	To evaluate diet	Mote Marine Laboratory	50	28 Completed Initial Analysis; 22 pending (plus 1999-2000 animals)

Toxicology	Identification and quantification of toxins including pesticides, organochlorides and heavy metals	National Institute of Standards and Technology Charleston, SC	33	26 Pending 7 completed for POPs
		National Ocean Service Center for Coastal Environmental Health and Biomolecular Research	5	1 Completed for water pesticides 4 Pending
Virology	Viral identification/ isolation to examine infectious processes	Carlos Romero, Ph.D. University of Florida	31	21 Completed 10 Pending

* Level 3: full examination including necropsy and sample collection; Level 2: no necropsy, but some samples collected (typically skin, blubber, muscle and/or teeth); Level 1: basic data collected including length, sex and location.

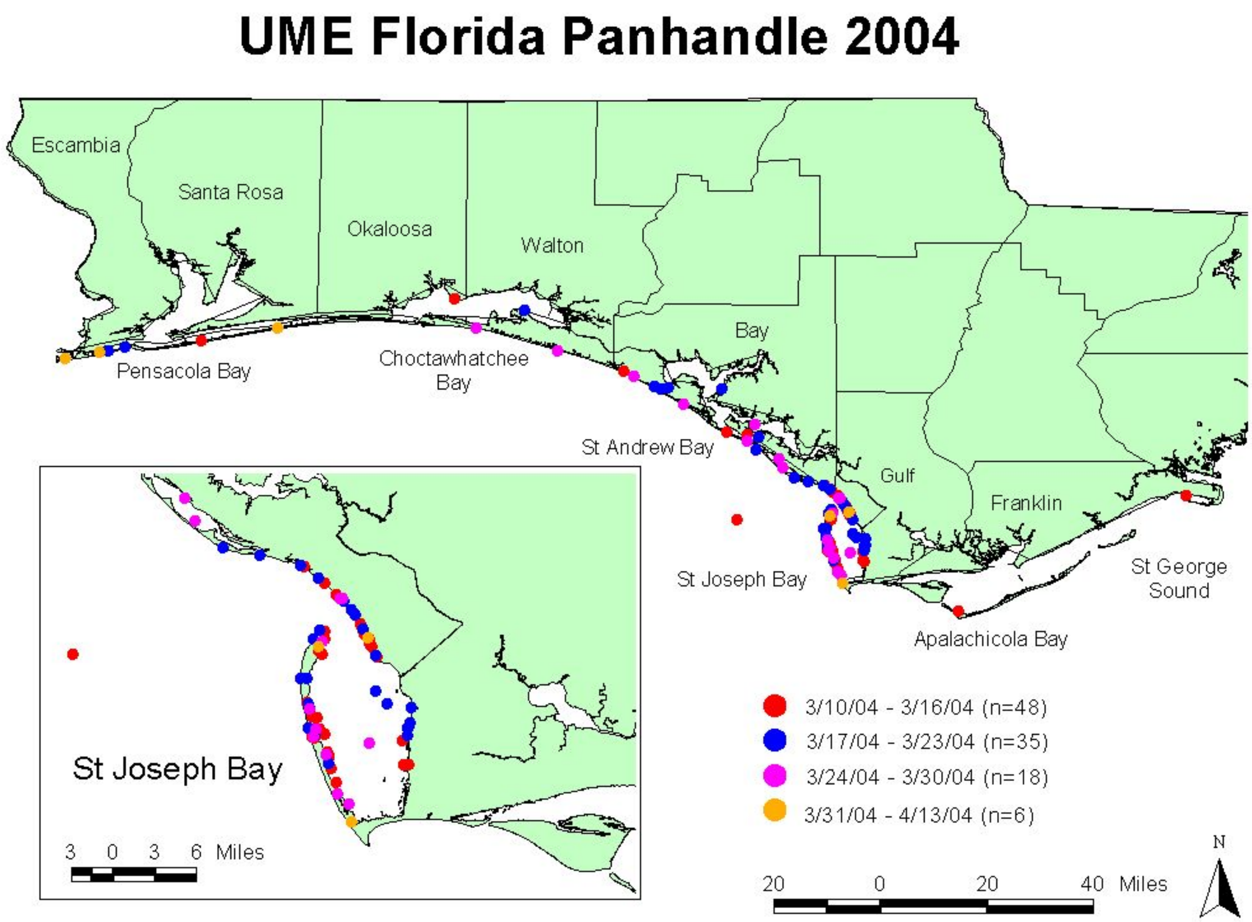


Figure 1. Map of the Florida Panhandle indicating locations where the 107 reported bottlenose dolphins were recovered. Insert depicts Gulf County and Bay County including St. Joseph Bay where the majority of animals stranded from March 10 - April 14, 2004.

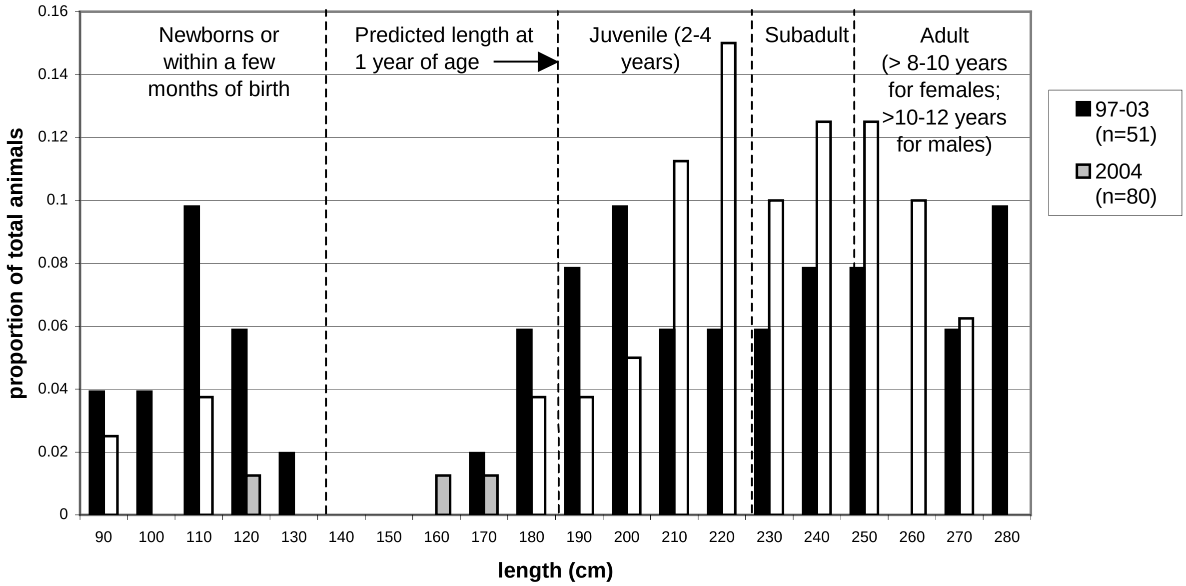


Figure 2. Standard length distribution (in cm) of 80 stranded dolphins recovered during the Florida Panhandle UME March-April 2004, divided into age categories based on length, and compared to length data from stranded animals reported in historical data (1997-2003).

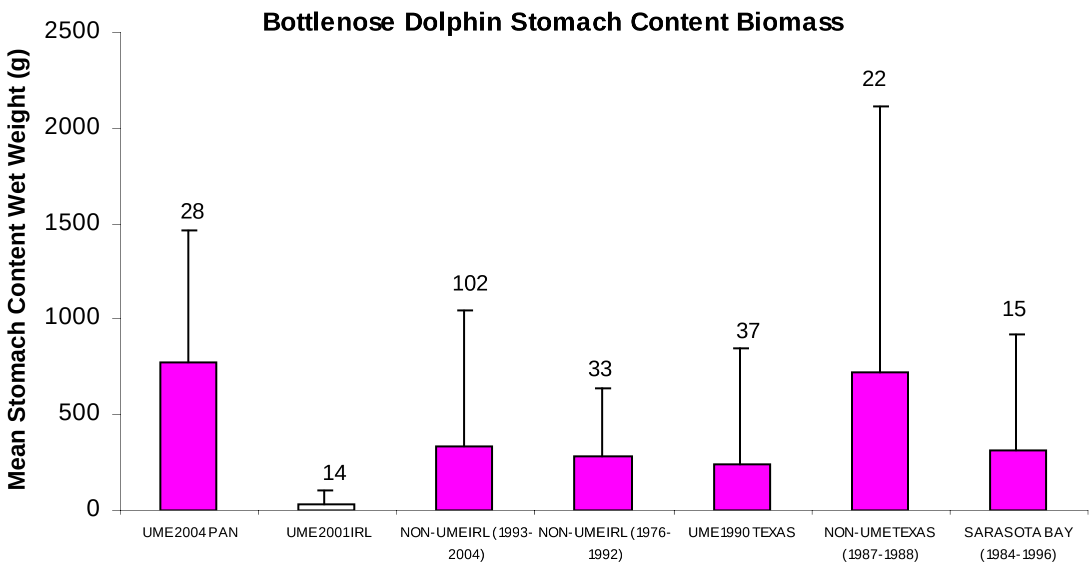


Figure 3. Stomach content biomass comparison.

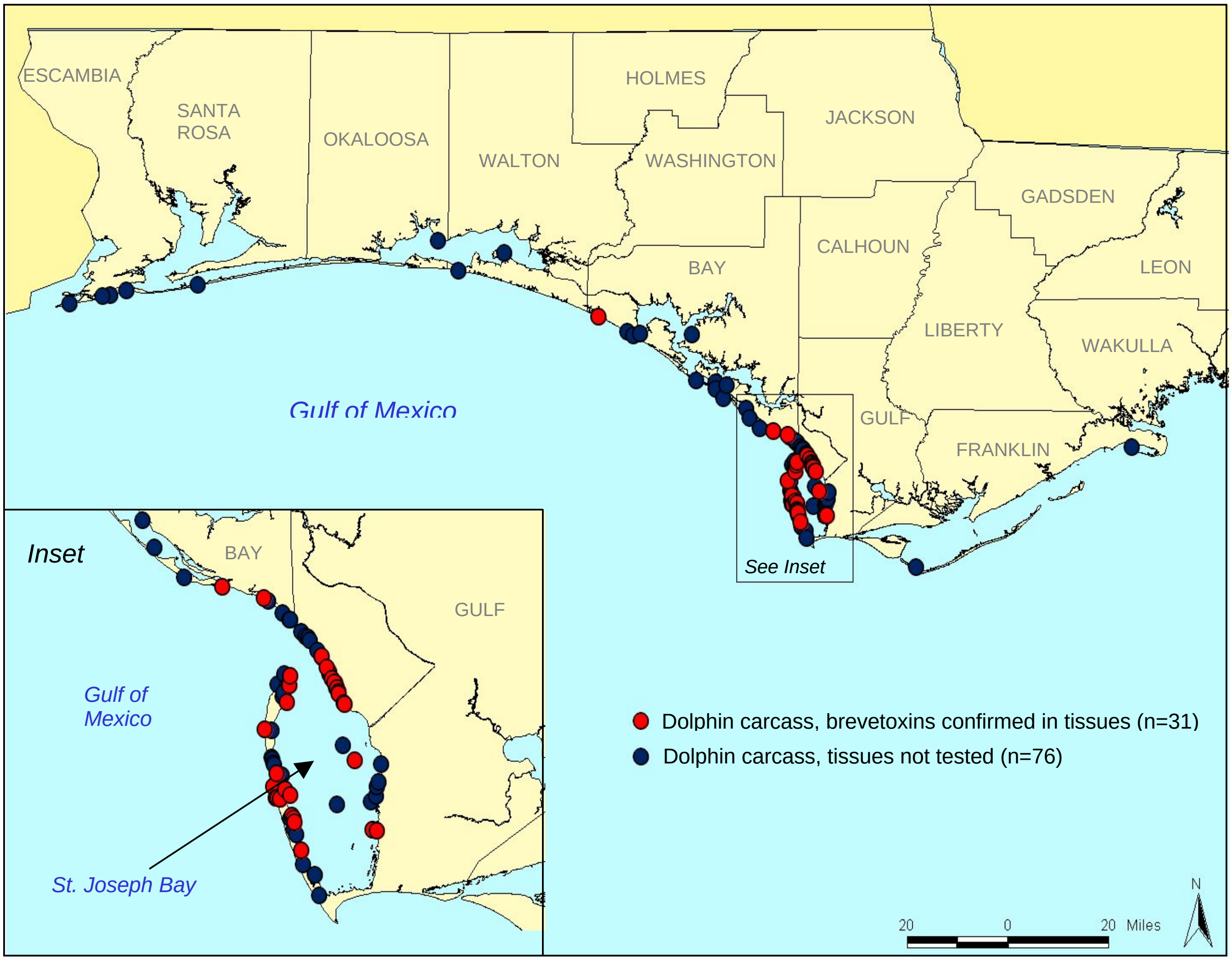


Figure 4. Dolphin Carcass Recovery Locations (n =107) in the Panhandle Region of Florida (March 10 – April 13, 2004)

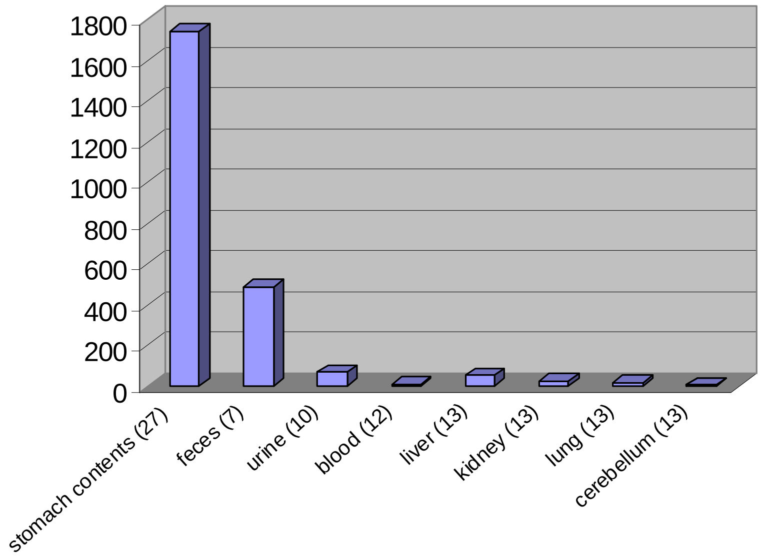


Figure 5. Brevetoxin concentrations found in stranded bottlenose dolphins. Concentrations in ng/g (tissues and feces) or ng/ml (urine and blood).

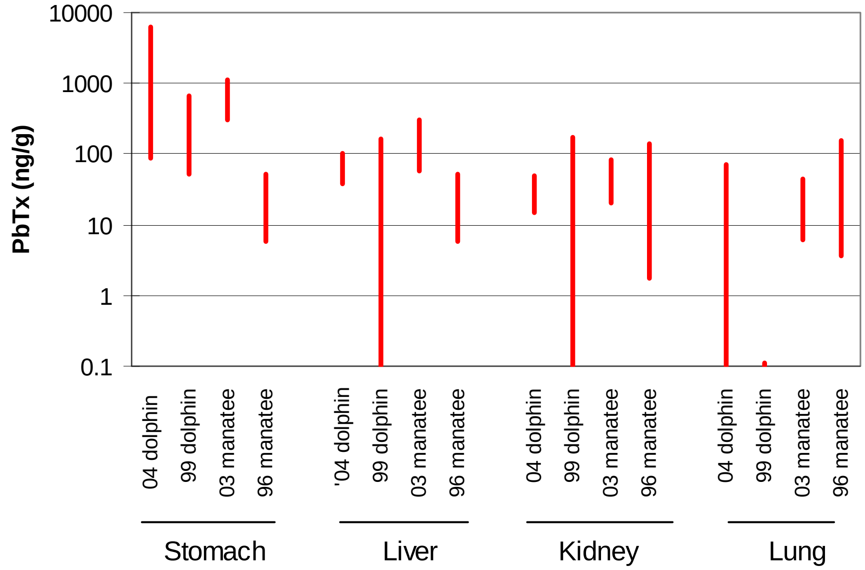


Figure 6. Ranges of brevetoxin concentrations found in tissues of bottlenose Panhandle dolphins ('99 and '04 mortality events) and manatees ('96 and '03 mortality events). Bars reflect high and low values for PbTx in ng/g.

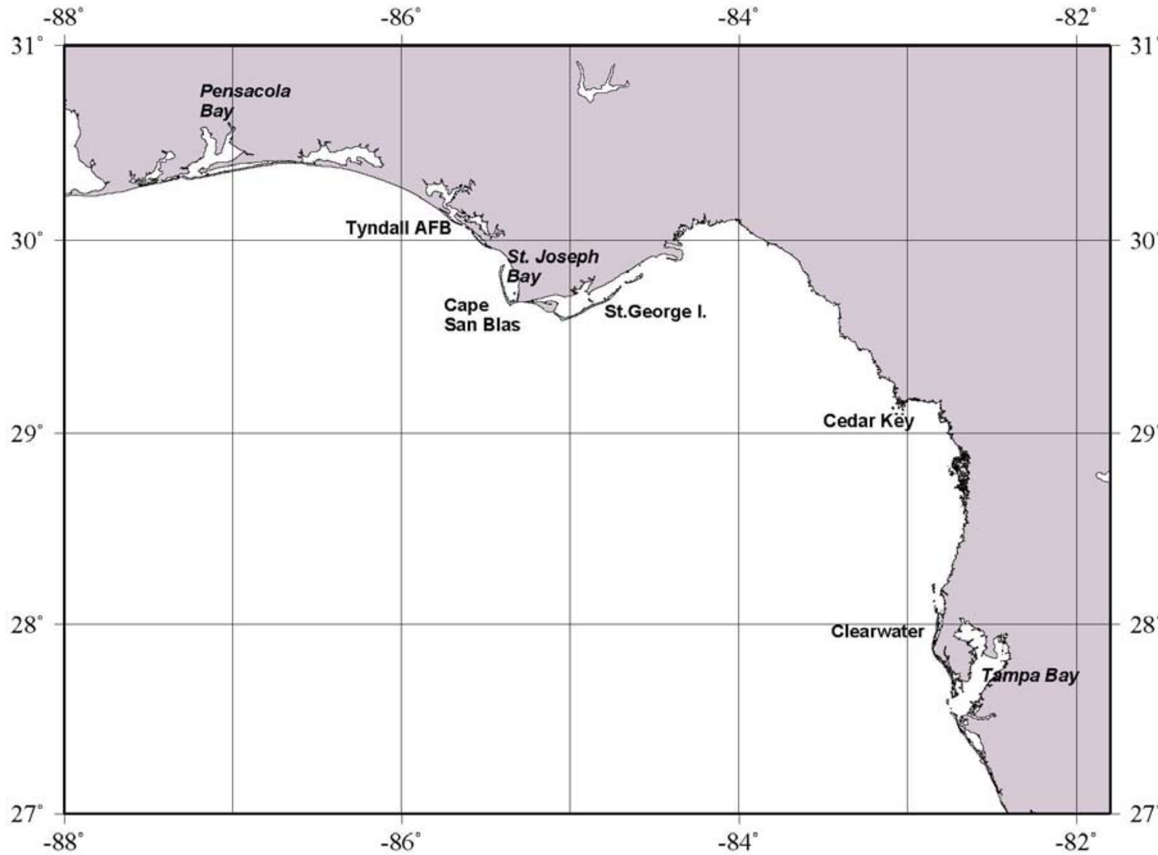


Figure 7. Map of Florida Panhandle indicating locations referenced in report.

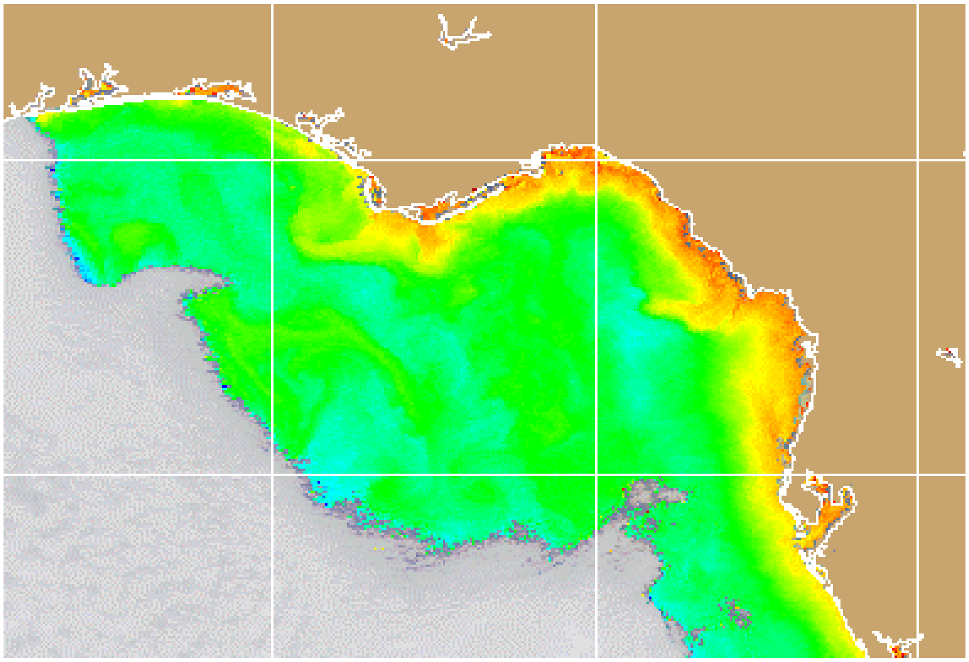


Figure 8. February 28, 2004, chlorophyll image. Yellows and reds show high chlorophyll (1-5 μg/L). High chlorophyll does not indicate the presence of harmful algae.

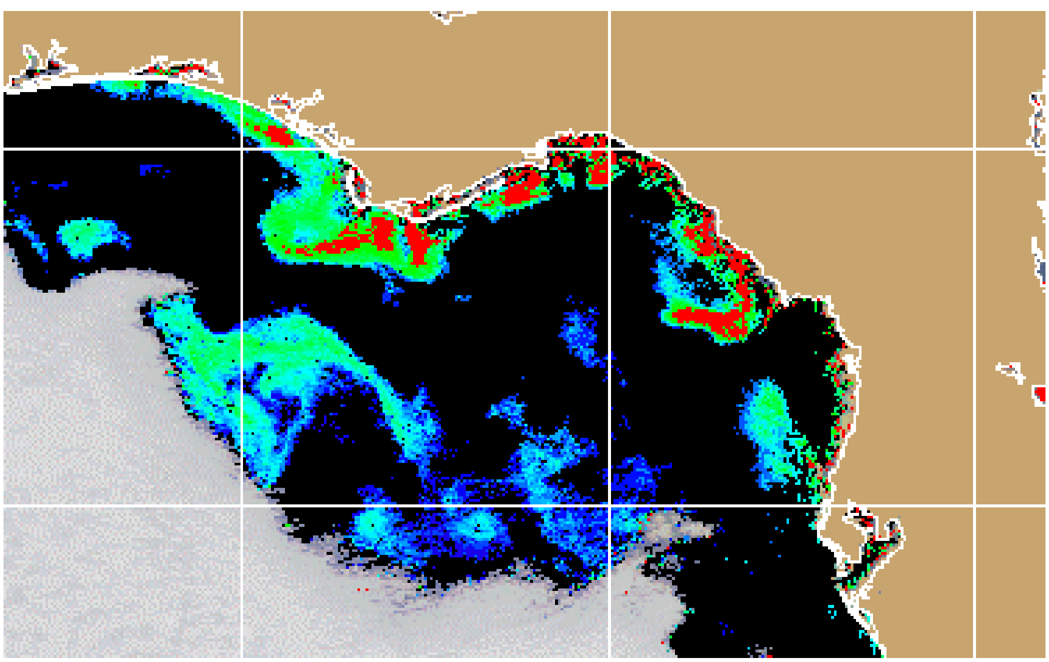


Figure 9. February 28, 2004 image showing new blooms in red. HAB events are more common as new blooms.

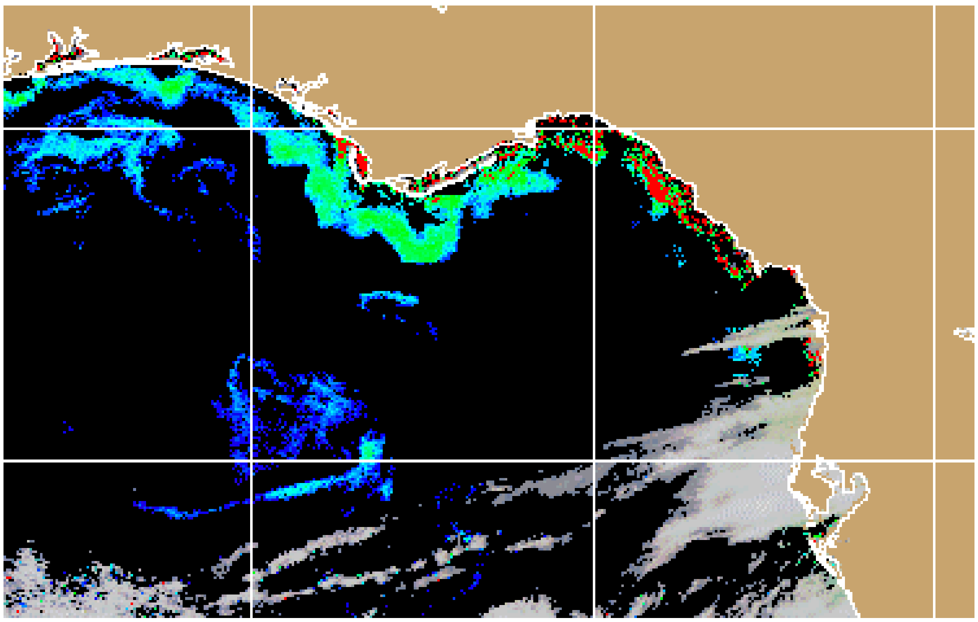


Figure 10. March 9, 2004 image showing the bloom in St. Joseph Bay.

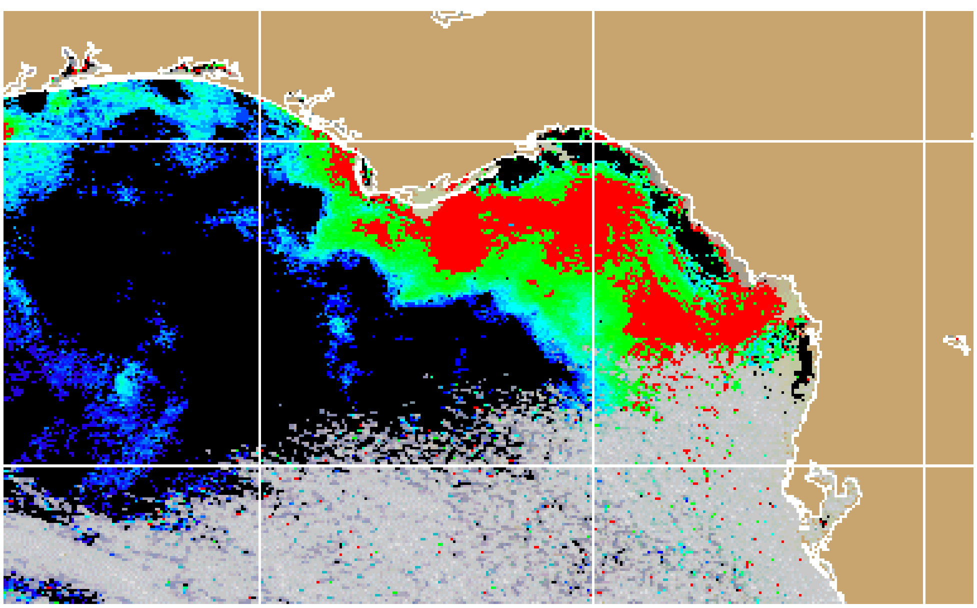


Figure 11. April 14, 2004 showing the apparent blooms all along the coast caused by the resuspension event. These apparent blooms occur because algae at the bottom in shallow areas are resuspended with the sediment.

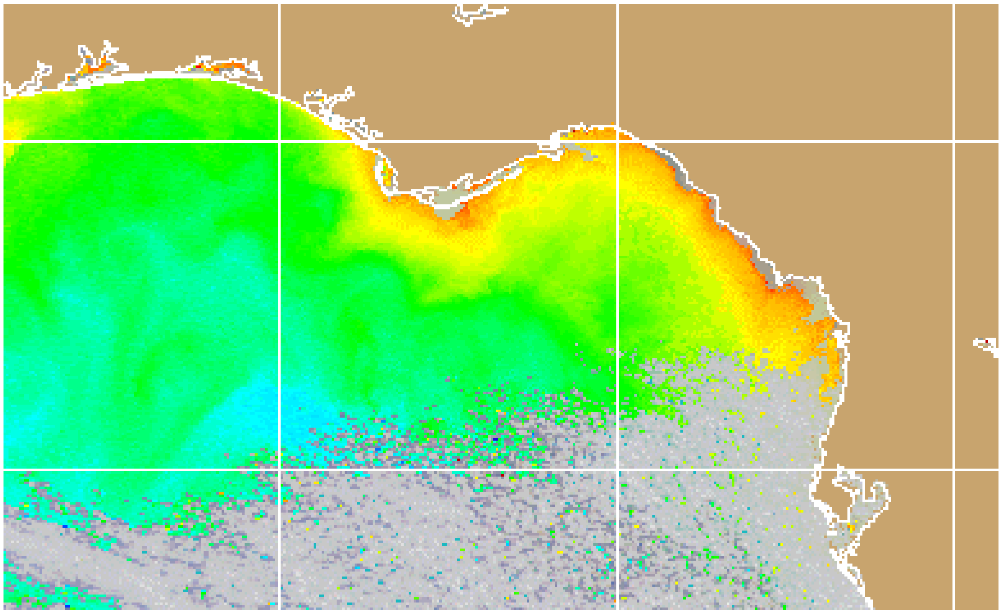
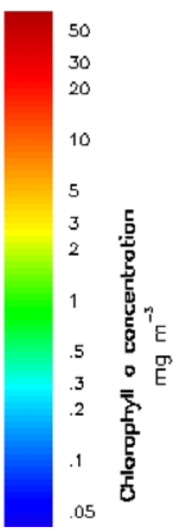


Figure 12. April 14, 2004, chlorophyll image.



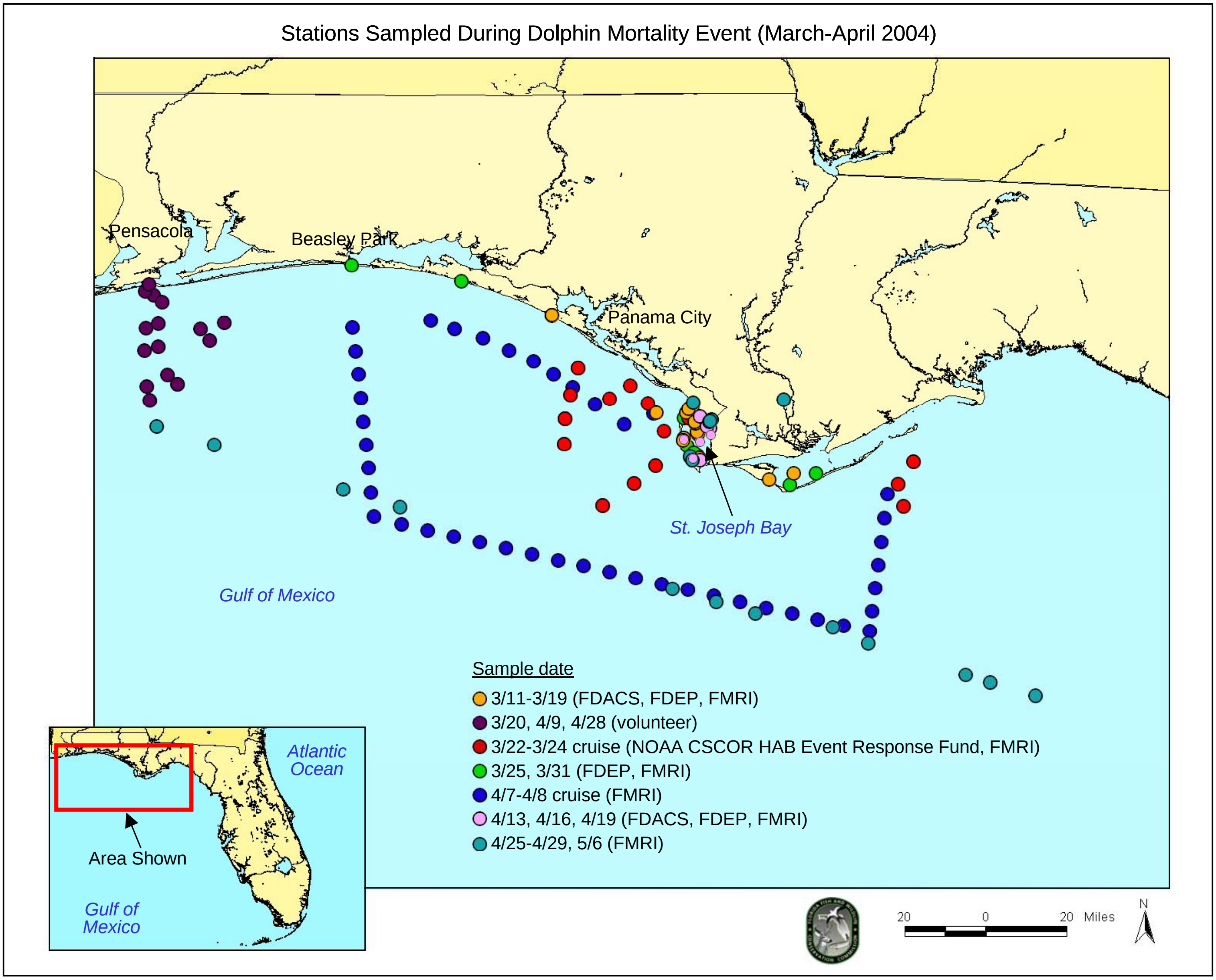


Figure 13. Stations sampled by FMRI or associated personnel during the dolphin mortality event, including the stations sampled during the NOAA CSCOR HAB Event Response Program sponsored RV Seminole Cruise (red dots).

VIII. APPENDICES

A. Criteria for Determining an Unusual Mortality Event

1. A marked increase in the magnitude of strandings when compared with prior records. There is no set formula for determining what magnitude would trigger a response. The NMFS Southeast Region has used a formula of the historic mean plus two times the standard deviation to determine a threshold level. The Working Group stated that magnitude must be weighed against other knowledge. As a pragmatic method, it was suggested that if a pulse in strandings is spread over an area or time frame that strains the capacity of the Stranding Networks to respond, it should be cause for concern.

2. Animals are stranding at a time of the year when strandings are unusual.

3. An increase in strandings is occurring in a very localized area (possibly suggesting a localized problem), is occurring throughout the geographical range of the species/population, or spreads geographically with time.

4. The species, age, or sex composition of the stranded animals is different than that of animals that normally strand in the area at that time of the year.

5. Stranded animals exhibit similar or unusual pathologic findings or the general physical condition (e.g., blubber thickness) of stranded animals is different from what is normally seen.

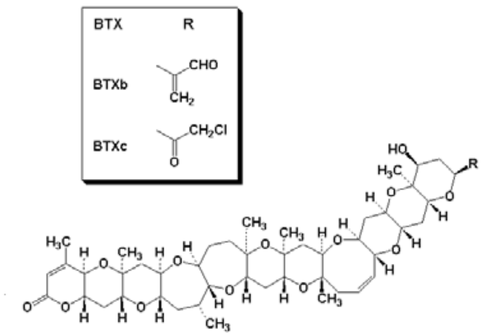
6. Mortality is accompanied by behavior patterns observed among living individuals in the wild that are unusual, such as occurrence in habitats normally avoided or abnormal patterns of swimming and diving.

7. Critically endangered species are stranding. Stranding of three or four right whales, for example, may be cause for great concern whereas stranding of a similar number of fin whales may not.

B. Fact Sheet on Brevetoxin & Florida Red Tides

Source of Brevetoxin (BTX): Red tides in Florida are caused by annual blooms of the dinoflagellate *Karenia brevis* which have been recorded since at least the mid 1800s. *Karenia brevis* is principally distributed throughout the Gulf of Mexico, with occasional red tides occurring along the mid and south Atlantic coast. The State of Florida continuously monitors for *K. brevis*, which produces nerve toxins (neurotoxins) known as brevetoxins that can cause serious public health effects and significant animal mortalities.

K*arenia brevis* Source: FWC-Florida Marine Research Institute



Brevetoxin Structure
Source. http://www.cfsan.fda.gov/~mow/brevotox.html

Toxin/Mode of Action: Brevetoxins are polyether ladder neuro-toxins that bind to voltage-sensitive sodium channels, an important protein structure of cell membranes. Binding results in persistent activation of neuronal cells, skeletal muscle cells and cardiac cells.

Human Health Syndrome: Neurotoxic Shellfish Poisoning (NSP) is caused by human consumption of molluscan (e.g. oysters, clams) shellfish contaminated by brevetoxins. NSP is characterized by paresthesia (tingling), reversal of hot-cold temperature sensation, myalgia (muscle pain), vertigo, ataxia (loss of coordination), abdominal pain, nausea, diarrhea, headache, bradycardia (slow heart rate), and dilated pupils.

Syndrome Distribution: Although shellfish poisonings were known in Florida since the 1880s, the cause was not identified as *K. brevis* until 1960. Shellfish monitoring to protect the public from NSP is successfully managed by the State of Florida and there have been no human fatalities attributed to NSP. New NSP cases may rarely occur when people consume unregulated shellfish species, when shellfish are illegally harvested, or when blooms expand to other unmonitored geographic areas. For example, in 1987, an entrained *K. brevis* red tide that originated off the Florida west coast was transported to North Carolina waters, causing 48 NSP cases.

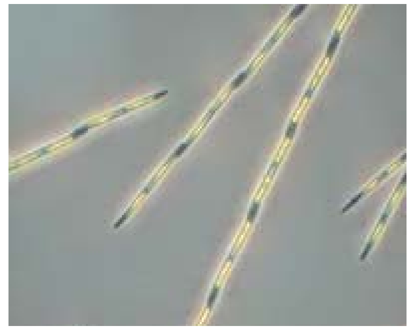
Other Human Health Effects: People can suffer from respiratory effects when brevetoxins become aerosolized through the disruption of *K. brevis* cells by breaking waves, surf or onshore winds. The first written report of respiratory irritation caused by a Florida red tide was in 1917.

Effects on Other Organisms: *Karenia brevis* red tides are responsible for the annual mass mortalities of thousands of fish. In some years, notable mass mortalities of marine mammals, birds, and sea turtles have been reported. During 1946-1947, one of the largest *K. brevis* red tide events on record occurred in Florida waters and was associated with catastrophic mortalities of bottlenose dolphins, sea turtles, and numerous fish species from Tarpon Springs to Key West (150 miles). Other marine mammal and bird mortality events with red tide links include:

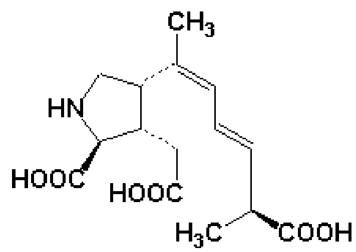
- Brevetoxin involvement suspected in the unprecedented die-off of more than 740 bottlenose dolphins, *Tursiops truncatus*, from June 1987-February 1988 along the eastern USA.
- During August 1999-February 2000, over 120 bottlenose dolphins stranded along the Florida panhandle during a co-occurring red tide.
- During an October 1973-May 1974 red tide, large numbers of birds were found moribund or dead, particularly double-crested cormorants, red-breasted mergansers, and lesser scaup.
- Substantial numbers of sick and dying cormorants co-occurred with red tide outbreaks along the west Florida coast during 1995-1999.
- *K. brevis* was implicated in mass mortalities of the endangered Florida manatee, *Trichechus manatus latirostri*s, in 1963, 1982, 1996, 2002, and 2003 when hundreds of animals died during the winter-spring in southwest Florida.

C. Fact Sheet on Domoic Acid

Source of Domoic Acid (DA): *Pseudo-nitschia*, a diatom genus, can be found worldwide. Diatoms are microscopic marine plants with silica shells. Several species of *Pseudo-nitschia* produce the toxin DA. These toxic species have been found on the east and west coasts of the US, as well as the Gulf of Mexico.



Chains of *Pseudo-nitzschia multiseries* Source: http://www.nwfsc.noaa.gov /hab/HABs_Toxins/HAB_Species/PseudoN/index.htm



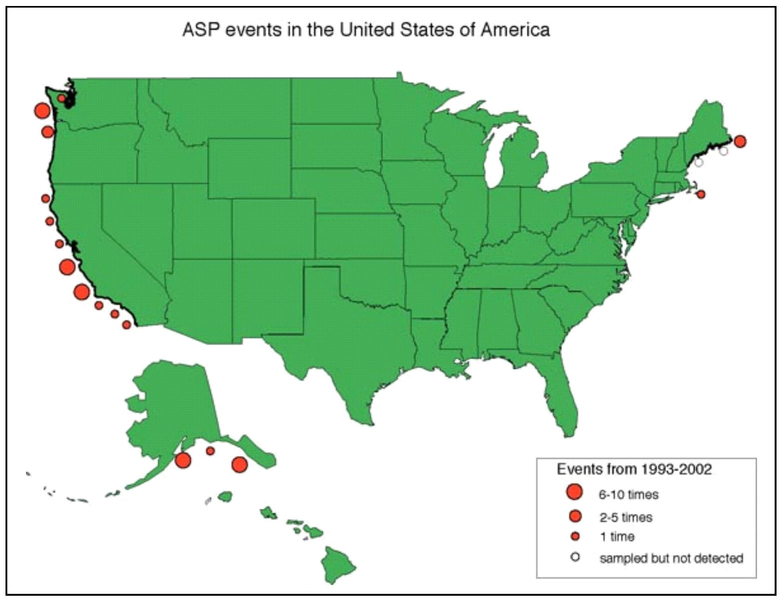
Domoic Acid Structure
Source: http://www.cfsan.fda.gov/ ~mow/domoic.html

Toxin/Mode of Action: DA is a tricarboxylic acid that acts as a neurotoxin. It binds to glutamate receptors, which are involved in memory processing. When receptors are excessively activated, as occurs with DA, damage to neurons leads to permanent loss of neurological function.

Human Health Syndrome: DA causes the syndrome known as Amnesic Shellfish Poisoning (ASP). Mild cases arise within 24 hours of consumption of contaminated shellfish. Symptoms include nausea, vomiting, diarrhea, and abdominal cramps. In more severe cases neurological symptoms occur which include headaches, hallucinations, confusion, short-term memory loss, respiratory difficulty, seizures, coma, and in extreme cases, death.

Syndrome Distribution: ASP was recorded for the first time off the Atlantic coast of Canada in 1987 when three deaths and over 100 confirmed cases of acute intoxications followed the consumption of cultured mussels. Milder cases have subsequently been reported.

Effects on Other Organisms: Since the 1987 human outbreak, DA has been identified as the causative agent in the mass mortality of pelicans and cormorants off of California, extensive die-offs of sea lions, dolphins, and otters off of California and whale deaths near Georges Bank. The toxin is transferred to higher trophic level carnivores via the food chain, usually from filter feeding fish or bivalves.



Source:http://www.whoi.edu/redtide/HABdistribution/ICESmap/USASP93-02.jpg

***Pseudo-nitzschia* and DA in the Gulf of Mexico**: *Pseudo-nitzschia* spp. are present, sometimes at very high abundance, in coastal waters of the Gulf of Mexico. There is evidence that their abundance has increased as a result of eutrophication from increased nutrient supply to some coastal areas. Although DA has been detected in natural plankton samples and in cultures of *Pseudo-nitzschia* isolated from the Gulf, no cases of human or animal illness or death due to DA have been documented in the Gulf of Mexico.