August 31, 2010

Mississippi Canyon 252

PREASSESSMENT PLAN TO DETERMINE POTENTIAL EXPOSURE AND INJURIES OF NESTING AND HATCHLING KEMP'S RIDLEY TURTLES

Approval of this Kemp's Ridley Turtle Nesting and Hatchling Plan is for the purposes of obtaining data for the Natural Resource Damage Assessment. Each party reserves its right to produce its own independent interpretation and analysis of any data collected pursuant to this work plan.

This plan was implemented consistent with existing trustee regulations and policies. All applicable state and federal permits must be obtained prior to conducting work.

Jennifer Boyce 8/31/10
Department of Commerce Trustee Representative: Date

Donna J. Shaver 9/3/2010
Department of Interior Trustee Representative: Date

9/7/10
Louisiana Trustee Representative: Date

Joyce Meley 8/31/10
BP Representative: Date

Mississippi Canyon 252

PREASSESSMENT PLAN TO DETERMINE POTENTIAL EXPOSURE AND INJURIES OF NESTING AND HATCHLING KEMP'S RIDLEY SEA TURTLES

Introduction

Potential impacts of MC 252 oil and dispersants on the Gulf coast population of Kemp's ridley sea turtles could range from mortality to sub-lethal stress and chronic impairment, including potential deleterious effects on reproduction and recruitment.

A Technical Working Group (TWG) composed of technical experts and trustee agency representatives has been assembled to draft a work plan to carry out reference (pre-discharge) assessment and post-discharge assessment (together, pre-assessment) of Kemp's ridley sea turtles along the Texas coastline of the Gulf of Mexico to support the Natural Resource Damage Assessment (NRDA) process established by the Oil Pollution Act of 1990 (OPA). BP has participated in a review capacity.

This Kemp's Ridley Nesting and Hatching Assessment Plan ("Plan") is part of the Pre-assessment Phase of the NRDA. All studies called for by the Plan were completed before the execution of the Plan because the Plan utilizes some of the ongoing efforts conducted on behalf of the Division of Sea Turtle Science and Recovery at the Padre Island National Seashore. In 1986, Padre Island National Seashore began systematic efforts to detect, investigate, and protect nesting by Kemp's ridleys and other sea turtles on North Padre Island, and these efforts have grown over the years. Nest detection patrols occur on the entire Texas Gulf of Mexico beachfront to some extent during the Kemp's ridley nesting season. Collection of Kemp's ridley nests this year occurred primarily between May and July. The data collected in this effort is similar to that being collected in the Loggerhead nesting NRDA plan that is ongoing and was approved by BP and Trustees in July 2010. The work described in this Plan is a subset of the overall program and includes collection of data that supplements the typical assessment completed annually. The data collected pursuant to this Plan will provide information to support a potential determination to conduct restoration planning under Section 990.42 of the Oil Pollution Act (OPA) regulations, and information that could be useful to the Trustees in performing future assessment activities. The Plan provides for data collection to document post-discharge conditions consistent with the standard operating protocols (SOPs) referred to in this document (see 15 C.F.R. §990.43).

Purpose:

The purpose of this Plan is to determine potential exposure to MC 252 oil and dispersants (hereafter referred to as "MC 252 oil") and associated injuries of the nesting adult Kemp's ridley sea turtles that reside in the Gulf of Mexico and nest along the Texas shoreline. This study is being conducted in partnership with a separate coordinated project to determine potential exposure and injury to adult loggerhead sea turtles that nest along the shores of the Gulf of

Mexico. In combination, these two studies aim to provide insights into the potential impact to nesting sea turtles from MC 252 oil along the entire Gulf coast of the United States.

Objectives:

1. Assess nesting female physical condition, inter-nesting movements, and blood chemistry as part of the normal annual assessment. In addition, the Plan calls for assessment of potential egg and hatchling toxicity impacts and hatching and emergence success as a function of pre-discharge, and post-discharge concentrations of MC 252 oil in and on nesting females, hatchlings, eggs, and nesting substrate to determine the potential relationship between MC 252 oil exposure and injury.

2. Assess possible toxicological and physiological effects and impairments in nesting females, eggs, and hatchlings along beaches in the Gulf of Mexico within Texas.

The intent is to achieve these objectives by conducting nesting female physical evaluations; satellite tracking of nesting female inter-nesting and post-nesting movements; collecting blood samples from nesting females; collecting tissue samples from eggshells, non-viable eggs, and hatchlings; and assessing hatching and emergence success along beaches within the study area. Samples will be analyzed for MC 252 oil (including PAHs) and physiological parameters.

Background:

Five species of sea turtles nest on sandy beaches along the Gulf of Mexico coast from the Dry Tortugas, Florida to southern Texas: loggerhead (*Caretta caretta*), green (*Chelonia mydas*), Kemp's ridley (*Lepidochelys kempii*), leatherback (*Dermochelys coriacea*), and hawksbill (*Eretmochelys imbricata*). Nearly the entire Gulf coast supports turtle nesting except for the Big Bend of Florida (Pasco north through to Wakulla Counties) which has no sandy beaches. Satellite tracking indicates turtles may travel and forage within the Gulf of Mexico, both during (inter-nesting movements) and between (migratory movements) nesting seasons, making nesting female turtles, eggs, and the hatchlings they produce, potentially susceptible to exposure and injury from the MC 252 oil spill (Seney and Landry 2008, Shaver and Rubio 2008). Because green, leatherback, and hawksbill turtles nest in relatively low numbers along the northern Gulf of Mexico, those species will not be included in this Plan. Due to logistical differences in management between loggerhead and Kemp's ridley turtles, this Plan addresses impacts to adult nesting Kemp's ridley turtles, eggs, and hatchlings. A separate NRDA plan addresses loggerhead turtles.

The Kemp's ridley sea turtle is comprised of one population and is listed as endangered under the Endangered Species Act (ESA). The majority of Kemp's ridley turtles remain in the Gulf of Mexico for their entire lives, and, consequently, the Kemp's ridley turtle is highly dependent on the Gulf of Mexico for its life cycle. Virtually all adults inhabit the Gulf of Mexico and nesting occurs almost exclusively on Gulf of Mexico shorelines. Post-nesting females and other life stages of Kemp's ridley turtles occur within the area of the MC 252 spill.

Most Kemp's ridley turtles nest along the northwestern Gulf of Mexico coastline in the state of Tamaulipas, Mexico. Nesting in the U.S. occurs from North Carolina to Texas. U.S. nesting is

concentrated in south Texas, with about half the Kemp's ridley nests recorded in the U.S. located at Padre Island National Seashore (PAIS) (Figure 1) (Shaver 2005, Shaver and Wibbels 2007). Due to years of bi-national, multi-agency conservation efforts, the Kemp's ridley population is increasing (National Marine Fisheries Service, U.S. Fish and Wildlife Service, and SEMARNAT, draft).

Female Kemp's ridley turtles nest approximately every 2 years and make reproductive migrations to breed and nest in the vicinity of their natal beach (Plotkin 2003). During the nesting season, females deposit 1 to 4 clutches of 40 to 140 eggs on the beach at approximately three-week intervals throughout the season. Turtles then travel back to their foraging sites, to which they also show a high level of fidelity (Plotkin 2003, Morreale et al. 2007, Shaver and Rubio 2008). These sites may be nearby or hundreds to more than a thousand kilometers away from each other. Kemp's ridley nesting females remain within the vicinity of a nesting beach or transit up to 200 km away during the inter-nesting period (Shaver and Rubio 2008). Males may remain resident off the nesting beach year-round (Shaver et al. 2005).

The possibility of MC 252 oil exposure in nesting Kemp's ridley females is supported by historical tracking studies indicating that Kemp's ridley turtles that nest along the Gulf of Mexico coast in Texas remain within the Gulf of Mexico during the inter-nesting interval and between nesting seasons (Seney and Landry 2008, Shaver and Rubio 2008). Shaver and Rubio (2008) tracked inter-nesting and post-nesting movements of Kemp's ridley turtles that nested on North Padre Island, Texas from 1997-2006 and documented migration routes and foraging areas for these turtles. They found that most of the turtles left south Texas waters after the nesting season was completed, traveled along the continental shelf, and were last found in waters offshore from the upper Texas coast, Louisiana, Mississippi, Alabama, or the west coast of Florida. Waters offshore from Louisiana were identified as high-use zones. Seney and Landry (2008) tracked adult females after they emerged for nesting on the upper Texas coast. They also documented usage of waters offshore from the upper Texas coast and Louisiana. For females nesting in 2010, movements after the nesting season may overlap with the extent of discharged MC 252 oil.

Exposure to crude oil and its derivatives has previously caused lethal and sub-lethal effects to adults, impacted egg production, and caused embryo mortality and hatchling deformities (Milton et al. 2003; Bell 2005; Bell et al. 2006; Van Meter et al. 2006). In addition, eggs exchange water and gases with the external environment possibly making them susceptible to physical and chemical effects of oil from oil-contaminated sand (Carthy 1996).

Kemp's ridley sea turtles can be exposed to oil through three primary exposure routes: dermal absorption, inhalation, and ingestion. Swimming in oil-covered water or underwater through dispersed oil will lead to direct dermal exposure to skin and carapace surfaces as well as the eyes. When turtles surface to breathe, they may be exposed via inhalation of the toxic volatile vapors emitted by oil on the surface as well as aerosols of oil resulting from surface activity. Ingestion exposure can occur through direct ingestion of oil or indirectly by ingestion of oil-contaminated foods. This pre-assessment effort is intended to determine if nesting females were exposed by measuring MC 252 oil contamination on the surface of the carapace, in blood samples, and in deposited eggs. Potential biological responses to MC 252 oil exposure will be documented by evaluating hatching and emergence success and signs of possible toxicity in

embryos or hatchlings (e.g. deformities). Blood samples may also provide hematological and clinical chemistry data for use in assessing possible responses of the hematopoietic, immune, renal, hepatic and other homeostatic systems.

General Study Approach

All nesting female Kemp's ridley sea turtles observed by biologists were tagged; however, nesting females were not observed at about half the nests documented in Texas. This lack of knowledge can be addressed by obtaining tissue samples from embryo mortalities within the nest for purposes of matching nesting females to as many nests of unknown maternity as possible (Frey et al. in press, Dutton et al. 2006, Frey et al. 2008). This analysis may be helpful in investigating potential transfer of MC 252 oil from nesting females to eggs.

Satellite tracking of adult nesting Kemp's ridley turtles will be used to determine their location during the inter-nesting and post-nesting intervals. These data will be overlaid with available information on the distribution of MC 252 oil to provide an evaluation of potential MC 252 oil exposure of nesting female turtles while at sea during the inter-nesting and post-nesting phases.

Scute samples were obtained from turtles outfitted with satellite transmitters and a randomized subset of the other females that nest on the upper Texas coast and at PAIS. Carbon and nitrogen isotope analysis will be conducted on these samples to help identify foraging strategies and potential for MC 252 oil exposure. Samples were collected from satellite tagged females that nested on the upper Texas coast and PAIS in the 2010 nesting season. These females typically arrive in the breeding area early in the season (March), thus they likely entered PAIS prior to any documented release of MC 252 oil.

A comparative analysis of previously collected data on nesting densities and frequency, hatching and emergence success on nesting beaches pre-discharge, concurrent-with-release, and post–discharge may provide an assessment of the potential injury resulting from MC 252 oil. Frequency and degree of deformities in hatchlings (dead and alive) and embryos will be visually assessed. Tissue from dead embryos and hatchlings, and choriallantoic membranes (CAMs) from hatched eggs from as many nests as can be obtained (approximately 50 nests) will be collected for assessment of potential MC 252 oil impacts in nesting females.

Study Area

Study areas include Kemp's ridley sea turtle nesting beaches in Texas (Figure 1). The two primary study sites for nesting female assessments encompass Bolivar Peninsula, Surfside, and PAIS in Texas. These sites encompass over 150 miles of beach and account for approximately 60 percent of the Kemp's ridley nesting activity in the U.S.

Sampling Design

Nesting Female Assessments:

The following description of the sampling design and proposed budget only represent efforts specifically associated with this Plan. Funding is requested from BP for activities that directly relate to and generate data to be used in the NRDA process. The required activities described in this Plan needed to be conducted in advance of formal signature of the Plan due to the timing of the nesting season. Pre-spill costs associated with this Plan were minimal and included training which would have been required in response to the spill. These efforts directly supported the NRDA effort even if they were completed prior to the spill date. The project manager for the annual assessment is not requesting any funds for her participation which have gone well beyond her normal responsibilities. If appropriate, the trustees will submit another work plan to address work that may be done in 2011 and beyond.

Unlike many sea turtle species, the Kemp's ridley is a daytime nester, rather than a nocturnal nester. Daytime surveys for nesting Kemp's ridley turtles were conducted throughout the entire Gulf of Mexico coastline in Texas. Surveys were conducted from March 15 through July 15, the period during which this species has historically nested on Texas beaches. The frequency and intensity of these surveys varied geographically from north to south. Following previously established research and survey programs developed by a variety of federal, state, and independent organizations, surveys were conducted weekdays from Bolivar Peninsula through Surfside, one to two days a week on Matagorda Peninsula, weekdays on Matagorda Island, once every eight days on San Jose Island, daily on Mustang Island, and repeatedly each day on North Padre Island (including PAIS), South Padre Island, and Boca Chica Beach. Surveys were conducted by vehicle (Utility Transport Vehicle (UTV) or four wheel drive SUV) and by foot, depending on the terrain. All nesting turtles encountered were fitted with metal Inconel flipper tags and a Passive Integrated Transponder (PIT) tag was inserted into the triceps muscle complex. Turtles were measured and any abnormalities or signs of injury (lesions, etc) recorded. A 10 cm square section of the highest point of the carapace was swabbed with a gauze pad and immediately placed in a pre-certified chemically-clean glass container. A 6 mm biopsy punch was used to obtain two tissue samples from the trailing edge of a rear flipper on each nesting female observed. One sample was placed in dimethylsulfoxide (DMSO) and the other was frozen.

Depending on availability of satellite tags and the number of turtles observed, up to 20 turtles located within the two primary study sites were fitted with Kiwi 101 satellite tags using established methods for sea turtle satellite telemetry (Seney and Landry 2008, Shaver and Rubio 2008). This included up to 10 turtles found nesting between Bolivar Peninsula and Surfside, Texas and up to 10 found nesting at PAIS (Figure 1). While Fastloc MK 10-AF satellite tags would provide higher resolution location data than Kiwi 101 satellite tags as well as dive time data, the Kiwi tags were used due to the limited availability of the Fastloc tags. Once a selected turtle had completed egg deposition, she was restrained and transferred to the laboratory for transmitter attachment. The tag was attached to the highest part of the carapace along the second vertebral scute using a two-part epoxy and resin adhesive. Once tagging was completed, the turtle was released and allowed to crawl back to the ocean within the boundary of the study site. The tag will provide data on turtle location, water temperature, day and time that the turtle's location is recorded, and an estimate of location accuracy. It should be possible for all tagged turtles to be tracked for a minimum of six months.

During tracking, movement and behavior will be characterized in relation to potential exposure to MC 252 oil, or the lack thereof. Movement data will be QA/QC'd for accuracy using standard techniques and analyzed in comparison to historical data. When feasible, approximately 10 milliliters of blood were collected from the cervical sinus of each nesting female turtle using vacutainer needles and tubes. Blood samples were partitioned for possible chemical and toxicological analysis. The analysis which may be conducted will be determined at a later date.

A 6 mm biopsy punch was used to obtain four scute samples (per approved FWS protocol) from each turtle that receives a satellite tag. Scute samples were also collected from up to 15 other adult females found nesting between Bolivar Peninsula and Surfside, or at PAIS. Analysis of scute samples will potentially provide data on food web positioning and likely food sources of nesting females.

Please note that as the initiation of this study occurred at the end of the 2010 nesting season, the full suite of adult samples could not be collected using the aforementioned techniques. Specifically, there were very limited, if any, opportunities to conduct carapace swabs or to collect blood samples in 2010.

Nesting emergence and success

All nest contents (unhatched eggs, hatched eggs, pipped eggs, and dead hatchlings) were collected from all nests and sent to the laboratory for processing and analysis as per SOPs. Dead hatchlings were visually inspected to assess possible obvious injury. When a nest was excavated, unhatched eggs were collected and placed in a polypropylene or glass container, and eggshells and CAMs from hatched eggs were collected and placed in a separate container. All samples were stabilized under refrigeration. Both types of residual nest material were collected in an unordered, random fashion, to avoid sampling bias. Eight eggs were withdrawn randomly from the total collection of unhatched eggs, and, following breakout into a clean, PAH-free acetone-rinsed Petri dish and determination of embryo stage and condition, their contents were pooled into a common chemically-clean amber sampling jar. This pooled egg sample will be sent to the laboratory for potential chemical, physiological and toxicological analysis to be determined.

Live and dead hatchlings and embryos were examined for gross deformities. Muscle and skin tissue were collected from up to five dead embryos and/or dead hatchlings from nests of unknown maternity and sent to the laboratory for processing and DNA kinship analysis.
As oil compounds, particularly the toxic components such as PAHs, are quickly metabolized and often difficult to document in exposed organisms, CAMs, the waste repositories of developing embryos, were salvaged from egg shells and unhatched eggs of all nests. CAMs will be pooled by nest and sent to the laboratory where they may be processed for chemical, physiological and toxicological analyses to be determined. A subsample of 10 CAMs, selected randomly from the pooled CAMs,were placed in a chemically-clean amber jar. Because other chemical contaminants can lead to toxicological effects similar to those caused by oil, a sampling of CAMs from nests that demonstrate deformities or unusually high hatchling failure (e.g.>50%) will be analyzed. This analysis is intended to eliminate uncertainty surrounding the possibility of

toxicological response to chemical contaminant other than MC 252 oil. In the event that elevated mortality and/or presence of deformities in embryos or hatchlings warrants assessment, tissue will be collected from approximately 50 dead embryos and dead hatchlings. Potential relationships between exposure and injury may be established using chemical, biochemical and toxicological analyses of the aforementioned samples. Potential toxicological response and nesting success parameters may be assessed as a function of chemical exposure, satellite tracking information, territory use prior to nesting, food web utilization and other exposure metrics.

Sand surveys

Systematic sampling and chemical analysis of sand matrices along the entire length of the Padre Island National Seashore was conducted as part of the base-line assessment process, which is separate from this Plan. This sampling was performed by both the National Park Service and the State of Texas and occurred during the Kemp's ridley nesting season, thus capturing a park-wide chemical profile for the nesting range for comparison with nest content chemical profiles, as needed. The data from this sand sampling effort will be used to help eliminate uncertainly about the source of any MC 252 oil that may be found in eggs (i.e., maternal versus egg chamber exposure) and, thus, which resources may have been injured in the process of transferring MC 252 oil to the eggs.

Sample and Data Handling

MC 252 NRDA chain-of-custody procedures will be observed at all times for all NRDA samples. Those samples were transferred with appropriate chain of custody forms and shipped to the appropriate laboratories for processing and analysis.

All field and laboratory data were collected, managed and stored in accordance with written SOPs. The appropriate training on particular equipment or in the conduct of specific field studies for all personnel involved with the project shall be documented and those records kept on file for the duration of this project. Unpublished data and published reports supporting this Plan will be made available to all parties.

Each laboratory shall simultaneously deliver raw data, including all necessary metadata, generated as part of this work plan as a Laboratory Analytical Data Package (LADP) to the trustee Data Management Team (DMT), the Louisiana Oil Spill Coordinator's Office (LOSCO) on behalf of the State of Louisiana and to ENTRIX (on behalf of BP). The electronic data deliverable (EDD) spreadsheet with pre-validated analytical results, which is a component of the complete LADP, will also be delivered to the secure FTP drop box maintained by the trustees' Data Management Team (DMT). Any preliminary data distributed to the DMT shall also be distributed to LOSCO and to ENTRIX. Thereafter, the DMT will validate and perform quality assurance/quality control (QA/QC) procedures on the LADP consistent with the authorized Analytical Quality Assurance Plan, after which time the validated/QA/QC'd data shall be made available to all trustees and ENTRIX. Any questions raised on the validated/QA/QC results shall be handled per the procedures in the Analytical Quality Assurance Plan and the issue and results shall be distributed to all parties. In the interest of maintaining one consistent data set for use by

all parties, only the validated/QA/QC'd data set released by the DMT shall be considered the consensus data set. The LADP shall not be released by the DMT, LOSCO, BP or ENTRIX prior to validation/QA/QC absent a showing of critical operational need. Should any party show a critical operational need for data prior to validation/QA/QC, any released data will be clearly marked "preliminary/unvalidated" and will be made available equally to all trustees and ENTRIX.

The Trustees and BP will work cooperatively to determine the appropriate chemical, physiological, and toxicological analyses to conduct on the samples gathered pursuant this Plan.

Principle Investigator:
Donna Shaver [redacted], National Park Service

Other investigators:
Kim Reich [redacted], Andre Landry [redacted] Texas A&M University at Galveston

Michael Hooper [redacted] U.S. Geological Survey, Columbia Environmental Research Center

Celine Godard-Codding [redacted] Texas Tech University

Thomas McDonald, West, Inc.

U.S. Fish and Wildlife Service Coordinators:
Tom Shearer [redacted]

Ann Marie Lauritsen [redacted]

Heath Rauschenberger [redacted]

Primary Responsibilities of Lead Investigators

Name	Role
Dr. Donna Shaver National Park Service	Principle Investigator/Project Coordinator
Dr. Kim Reich Texas A&M University at Galveston	Lead Investigator for Bolivar Peninsula to Surfside
Dr. Andre Landry Texas A&M University at Galveston	Co-Lead Investigator for Bolivar Peninsula to Surfside
Dr. Michael Hooper USGS	NRDA, Restoration and toxicology Consultant
Dr. Celine Godard-Codding Texas Tech University	Toxicology consultant
Dr. Thomas McDonald West Inc.	Statistical consultant
Tom Shearer US Fish and Wildlife Service	Trustee representative, consultant on permitting
Ann Marie Lauritsen US Fish and Wildlife Service	Trustee representative, consultant on workplan development
Dr. Heath Rauschenburger US Fish and Wildlife Service	Trustee representative, consultant on toxicology

Sample Collection Summary Outline

Turtles

- Collect blood from nesting females
- Satellite track inter-nesting and post-nesting movements
- Scute sample for stable isotope analysis
- Collect tissue sample
- Carapace swipe

Nests

- Collect tissue samples from all nests, eggs, and dead hatchlings
- Collect CAMs from unhatched eggs and egg shells after hatching (all nests)

Nesting data

- Collect GPS points for every nest in project site

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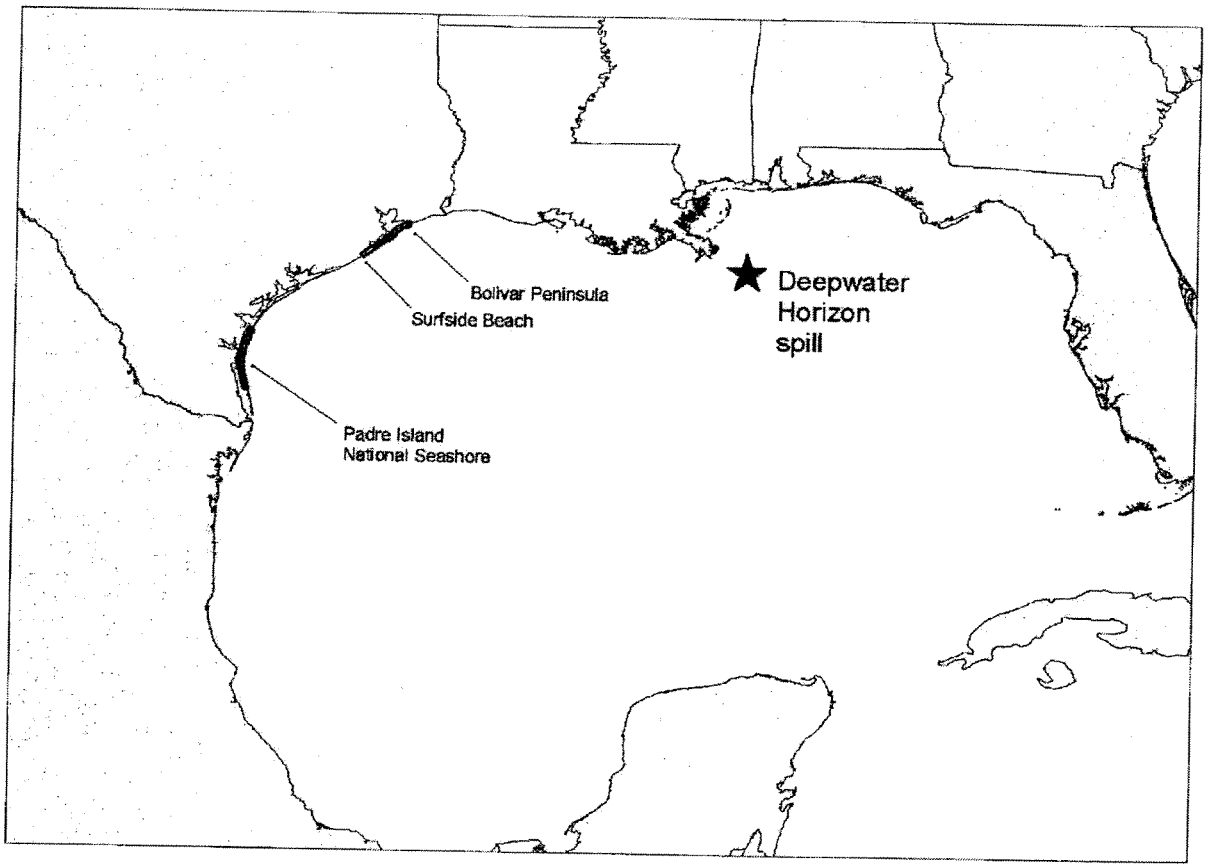

Figure 1. The Bolivar Peninsula through Surfside Beach, and Padre Island National Seashore are the two primary study sites in Texas where potential oil impacts and injury to nesting Kemp's ridley turtles from the Deepwater Horizon spill were assessed. Possible impacts to loggerhead turtles nesting along the shores of the Gulf of Mexico are also being assessed using similar methodology and that work is described in a separate NRDA Work Plan.

Proposed Budget for Kemp's ridley Nesting Study

Item	Unit	Year One
OVERALL PROJECT		
Program Manager: (GS -13) No salary requested		
Survey Coordinator: (GS 11 for 5 months with overtime)	$35/hr	63000
Technical Coordinator: (1.5 FTEs, GS 6 with overtime)	$22/hr	68000
Travel for Program Manager (for work group participation and information transfer for a full year)	$1000/mo	12000
SUBTOTAL		**$143,000.00**
PERSONNEL		
Monitoring to Identify nests, encounter nesting females and obtain samples		
2 technical experts to collect and process specific tissues (Texas Tech)		75000
8 field personnel for 5 months (GS 4 with overtime)	$20/hr	171600
Camp perdiem for field personnel ($50/day per person - approximately 300 person days over 5 months)		15000
SUPPLIES		
Lockable upright freezer with backup generator (-20c Freezer)		23000
100 PIT tags @$8 each, including injector applicator		800
200 metal tags @ $70 box of 100, including applicator pliers		140
10 KIWI 101 PTT tags	2000	20000
ARGOs Satellite time for 10 tags	1500	15000
Biopsy supplies, consumables for needles, cryotubes, etc		500
Sample collection containers for tissues , vials, gloves,		1500
Liquid Nitrogen Dewar, coolers		3600
Fedex shipping		2000
SUV to transport nests to incubation facility		35000
Fuel - $1200 a month x 5 months		6000
2 UTVs - Yamaha Reno 4x4 or equivalent	10,500	21000
Padre Island SUBTOTAL		$390,140.00
Padre Island TOTAL		**$533,140.00**