

**Assessing Potential Sublethal and Chronic Health Impacts from the
Mississippi Canyon 252 Oil Spill on Coastal and Estuarine Bottlenose
Dolphins**

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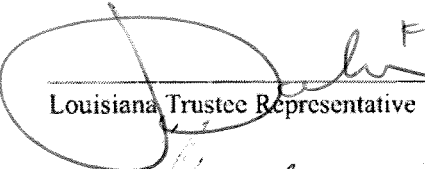
**Assessing Potential Sublethal and Chronic Health Impacts from the Mississippi Canyon
252 Oil Spill on Coastal and Estuarine Bottlenose Dolphins**

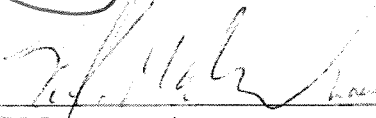
Approval of this work plan is for the purposes of obtaining data for the Natural Resource Damage Assessment. Each party reserves its right to produce its own independent interpretation and analysis of any data collected pursuant to this work plan.

The trustees have developed a preliminary conceptual model of the DWH release, potential pathways and routes of exposure, and potential receptors. This preliminary model has informed the trustees' decision to pursue the studies outlined in the work plan. By signing this work plan and agreeing to fund the work outlined, BP is not endorsing the model articulated in the work plan.

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

 FOR ROB RUCKER
Department of Commerce Trustee Representative 4/8/11
Date

 FOR ROLAND SUNDAY
Louisiana Trustee Representative 4/12/11
Date

 LAWRENCE McLEAN
BP Representative April 8, 2011
Date

Background:

Natural Resource Damage Assessment (NRDA) pre-assessment efforts documented oil exposure of bottlenose dolphin (*Tursiops truncatus*) stocks in Barataria Bay, Chandeleur Sound and Mississippi Sound. Dolphins were observed in oiled areas and some dolphins were observed with oil patches on their skin. No overt signs of distress were observed in association with oil exposure. However, possible sublethal or latent effects, such as organ damage and immune dysfunction, would not be detectable by the photographic and remote biopsy studies conducted to date. In addition to the possible chronic health effects from acute exposure, the dolphins may also be subject to adverse effects if oil and associated chemicals persist in the marine environment, including the marine food web. Other potential indirect impacts from potential habitat degradation and loss of prey resources are also of concern and may reduce survival and reproduction over the longer term [1, 2]. Such effects have the potential to impact the sustainability of stocks or communities [3].

Project Description:

This project will conduct capture-release health assessments of bottlenose dolphins in an impacted area (Barataria Bay, LA) and a reference site (Sarasota Bay, FL) to address potential sublethal, chronic and indirect health impacts of the Deepwater Horizon (DWH) oil spill. The capture-release project will involve a team of veterinarians, biologists and wildlife epidemiologists working together to conduct comprehensive health evaluations. The health assessments will enable researchers to directly assess potential injury endpoints (anemia, organ damage, immune suppression, endocrine disruption, indication of nutritional and chronic stress) in individuals and among cohorts. Additionally, the capture-release operation will allow for attachment of satellite and VHF tags to dolphins in Barataria Bay to better understand their movements, range and preferred habitats, which may assist in exposure assessment and restoration planning.

Asset requirements:

The sampling in Barataria Bay will require the use of 4-5 NOAA small vessels including 2-3 Zodiac RIBs and the R/V Megamouth which was specially designed for dolphin capture-release operations. The R/V Megamouth is a 30 ft. Munson Packman monohull with a bow ramp that is deployable to below the water line to facilitate loading and unloading of animals. There is ample space forward for animal processing and a protected area aft, suitable for basic laboratory procedures. In addition to the NOAA vessels, 2 small vessels will be provided by Louisiana Department of Wildlife Fisheries (LDWF) for the capture-release activities. LDWF will also provide a small vessel for follow-up tracking of tagged dolphins. The Chicago Zoological Society (CZS) will provide appropriate and well-tested vessels for the sampling in Sarasota Bay, including a 28-ft Tremblay, specially outfitted for dolphin capture-release research, with a removable transom providing access to a large, covered and padded veterinary examination deck.

Objectives:

The objectives of this Plan are to:

- Provide information on potential health impacts of DWH oil and dispersants in bottlenose dolphins.
- Gain a better understanding of movements and ranges of dolphins in Barataria Bay, an area which has been impacted by oil from the MC 252 oil spill.

This Plan will provide estimates of potential injuries for dolphins in Barataria Bay potentially exposed to MC252 oil.

The capture-release health assessment activities in Barataria Bay will be conducted under NMFS Permit No. 932-1489-09 issued to Dr. Teri Rowles. The study will employ protocols approved by the NOAA/NMFS ad-hoc Institutional Animal Care and Use Committee (IACUC) in 2009, and protocols will be reviewed by the Southeast Regional IACUC and NMFS permits office prior to capture fieldwork. Capture-release activities in Sarasota Bay will be conducted under NMFS Permit No. 522-1785 or pending renewal issued to Dr. Randall Wells and protocols approved by the Mote Marine Laboratory IACUC.

Methods and Operations:

Two sites (one where oiling occurred and one reference) will be targeted for health assessment efforts: Barataria Bay, Louisiana and Sarasota Bay, Florida. Barataria Bay, near Grand Isle, Louisiana, has been a focal site for the NRDA dolphin pre-assessment and is an area that has been impacted by oil. Due to the oiling, this is an area for which there is a potential for health effects from oil exposure and associated chemicals in estuarine dolphins. In addition, the Bay is relatively sheltered and has a number of shallow areas (< 1.5 meters) that would be favorable for setting a net to capture dolphins.

Sarasota Bay, Florida was chosen as a reference site because it is a Gulf of Mexico site that was not subject to visible oiling. In addition, some sampling of the Sarasota Bay dolphins (N=12) to establish pre-MC 252 oil spill baselines was conducted in May, 2010 under a grant from the Morris Animal Foundation. The efforts under this Plan will build on 2010 sampling efforts, increasing the sample size.

Approximately 15 dolphins will be captured, examined and released from Sarasota Bay within a 1 week period in May, 2011 and 30 dolphins from Barataria Bay within a 2 week period between June and October, 2011. Final dates for the Barataria Bay fieldwork will be determined based on availability of key personnel and resources. Due to their size (400-500+ lbs) and their protected status under the Marine Mammal Protection Act (MMPA), capturing and restraining dolphins without injuring either the animals or field personnel is challenging. The co-investigators for this effort bring extensive experience with capture-release health assessment methods and have been involved in a number of studies across the southeastern U.S.[4-8].

The health assessment will include a complete physical examination including cardiovascular assessment, examination of eyes, oral cavity and skin condition, and diagnostic ultrasound, as well as the following sample collection and analysis:

- Blood - hematology, serum biochemistry, endocrinology, chemical analysis, functional immunology
- Skin/Blubber -Analyses to be conducted under a separate work plan that BP and the Trustees agree to use best faith efforts to develop cooperatively.

- Urine – urinalysis, PAH metabolites

A more detailed list of health measurements is given in Table 1, and chemical analytes are listed in Table 2. Published reference intervals exist for hematology and blood chemistry parameters and references will be provided to the Trustees and BP or its representatives before fieldwork begins. Reference intervals (95th percentiles) for hematologic and serum biochemical parameters have been estimated based on pooled data (N=255) from dolphins in 4 southeastern US coastal sites [9]. When the derived reference intervals were applied independently to the 4 sites, the numbers of out-of-range data points were consistent with the expected number (2.5% below and 2.5% above), indicating that the values are applicable across different geographic areas [9]. Data for comparison of endocrine and functional immune measurements also exist from previous dolphin health assessment studies conducted in Sarasota, Florida; St. Joseph Bay, Florida and Beaufort, North Carolina [10, 11]. Similar comparisons have made for other bottlenose dolphin populations and have successfully identified population health issues [5, 12].

Additional samples for analyses unrelated to the oil spill injury assessment (*e.g.*, urine and feces for analysis of algal toxins) that will help to establish baseline information for Gulf of Mexico estuarine dolphin stocks relevant for NOAA or CZS research objectives will also be collected but are not listed in detail here and are not included in the cost estimate for this injury assessment.

Specific Procedures

Capture: Dolphins will be captured using established methods [4-8, 13]. The capture fleet will consist of 6-7 boats: a net boat, up to five chase boats, and a veterinary processing boat. The crew will include a Medical Safety Officer to oversee safety of the operations and to provide emergency medical assistance if necessary. Capture operations will adhere to safe boating regulations and all staff will be briefed on safety protocols prior to going out onto the water.

Boats will search for dolphin groups, concentrating search efforts in areas identified as optimal potential capture locations. The identification of optimal areas will be based on water depth, bottom substrate, avoidance of high boat traffic, and likelihood of finding dolphins based on dolphin sightings from previous photo-identification surveys (Figure 1). To date there is no evidence to suggest a bias in areas of usage (*i.e.* that unhealthy or potentially more highly exposed dolphins would be more likely to be found in any given area), but efforts will be made to spread sampling locations across multiple areas. However, safety of people and dolphins will be the first consideration in choosing areas to set the net.

A dolphin group will be followed until it reaches a location where the net (366-500 m long, 4-7 m deep, 22 cm stretch mesh seine net with a float and single or double lead lines, depending on the site) can be safely deployed and personnel will be able to safely handle captured dolphins. The goal will be to capture small groups of dolphins (<5) in shallow (<1.5 m) water, over solid to semi-solid substrate in protected areas with little to no current. Alternatively, 1-2 dolphins can be captured in deeper water, using modified techniques. In the capture process, the net boat encircles the targeted dolphins at high speed while deploying the capture net astern. One of the chase boats circles where the net “compass” was begun, to ward dolphins away, while the remaining chase boats take up station around the compass, maintaining clearance so as not to impede the net boat. Once the compass is closed, chase boat crews will either deploy around the outside of the net (in shallow water) or will remain on their boats (in deep water). If dolphins become entangled in the net shortly after being set, handlers can respond to and support these animals. If the encircled dolphins do not become entangled of their own accord, the net boat and

dolphin handlers can reduce the size of the compass to force dolphins to entangle themselves. Once dolphins become entangled, they are restrained and supported by handlers and then removed from the net. Captured dolphins are then processed, both in the water and on-board the processing vessel. Procedures have been organized to minimize the amount of time an animal spends out-of-water and standardized data collection protocols established by previous dolphin health assessments will be used [5, 14]. Each dolphin is released once processing is completed for that animal unless there is a reason to release animals together (socially dependent mother/calf pair). If there is a reason to release together, then the animals are processed sequentially and held in water until a dual release is possible.

BP or its' representative will be provided an opportunity to observe each dolphin sampling event that satisfies NOAA NRDA safety guidelines and permit requirements. The Trustees agree to make a good faith effort to provide BP or their representative with an opportunity to observe all aspects of the health assessment.

Health Evaluation: Blood will be drawn from fluke vessels through sterile procedures while the dolphin is held in the water and the fluke is elevated above the surface. Blood sampling and handling will follow quality assurance/quality control protocols developed by the National Institute for Standards and Technology (NIST). For females, an ultrasound examination will be conducted to assess potential pregnancy. Pregnant animals will not be taken aboard the processing boat and instead will be given an abbreviated in-water exam. For early term pregnancies, a more complete in-water exam may be given at the discretion of the lead veterinarian. Non-pregnant females and males will be placed in a sling, weighed and hoisted aboard the processing boat for further evaluation.

The complete examination will include morphometric measurements, sampling of urine feces, blowhole and blubber as well as a tooth extraction to determine age (as necessary, most dolphins in Sarasota Bay are of known-age because they have been followed since birth). Each dolphin will be photographed and branded with a 3-digit alphanumeric code on either side of its dorsal fin. Brands provide a long-term identifier on the dolphin's dorsal fin to aid in recognition during monitoring surveys [15]. These brands will provide life-long identification of individuals for mark recapture or other studies. Photos will enable investigators to match evaluated dolphins with those identified under the prior work plan for estuarine dolphin injury assessment.

Telemetry: Dolphins (other than dependent calves) captured in Barataria Bay will be tagged with both a single pin satellite-linked tag and a VHF radio tag. Both tag types will be applied along the trailing edge of the dorsal fin in such a manner as to not impact the body by rubbing, or cause early release due to increased drag or turbulence. A similar single pin attachment design for satellite-linked tags was successfully employed in a 2009 study of bottlenose dolphins off the coast of Georgia [16] and their use has already been reviewed by the Mote Marine Laboratory IACUC and approved for tagging of free-ranging dolphins and rehabilitated stranded dolphins. Satellite-linked tags which provide location data will be configured to transmit for 2-4 hours per day during prime satellite coverage periods which should allow for a 2-3 month tracking period. The VHF radio tags will enable acquisition of individual dolphins and provide a method for relocating the animal should the satellite-linked tag unexpectedly cease to function or if there are other indications/observations that the animal is in distress. As part of the follow up of these

animals, photo-identification surveys (lasting 2-3 days each) will be conducted by LDWF at 1-week, 3-weeks, 5-weeks and 10-weeks post-capture to assess biopsy healing and document tag loss as well as evaluate the overall visual appearance of tagged animals.

Sample analysis: Samples for hematology, serum biochemistry and endocrinology will be sent to Cornell University, Animal Health Diagnostic Laboratory (AHDL) for analysis in order to maintain consistency with prior dolphin health assessments and allow for comparison with existing reference intervals [9]. Functional immunology will be conducted by the laboratory of Dr. Sylvain De Guise at University of Connecticut to maintain consistency with data from prior dolphin health assessments in the Gulf (Sarasota and St. Joseph Bay, FL) and Beaufort, NC. Dr. De Guise's laboratory provides expertise in immunotoxicology and extensive experience in the conduct and interpretation of functional immune assays for cetaceans [5, 17-22]. Analysis of teeth for age determination will be performed by Wayne McFee (NCCOS) when teeth are obtained (*i.e.*, not in pregnant animals or animals of known age) using established methods [23, 24].

Analysis of blubber or skin samples for chemical contaminants, genetics, and hormones will be included in a separate sample analysis plan or plans, along with the analysis of remote biopsy samples, and is therefore not included in this Plan. Blubber surgical biopsies will be divided and subsamples will be archived for analyses to be determined under future plans. Surgical biopsy wedges are around 2.5 grams and should provide sufficient tissue for multiple subsamples. Under this Plan, blood samples will be analyzed for polycyclic aromatic hydrocarbons (PAHs), as well as additional persistent organochlorine contaminants (suite of persistent organochlorine pesticides, polychlorinated biphenyls and polybrominated diphenyl ether (PBDEs)). PAHs may not be present at elevated concentrations in the blood or blubber as they are rapidly metabolized by vertebrate species, most likely via induction of the mixed-function oxidases to more polar compounds in the liver. The PAH metabolites are then concentrated in the bile for elimination [25]. However, if the dolphins have been exposed to a large amount of petroleum and the liver enzyme system is overwhelmed such that it cannot efficiently metabolize the PAHs, there is the possibility that parent and alkylated petroleum-related PAHs will be measureable in concentrations well above background levels in tissues such as blood or blubber. The additional organochlorine contaminants are included in the proposed analyses as many of these compounds (*e.g.* PCBs) may exert toxicological effects similar to the PAHs and are thus important to include as potential confounding variables. The chemistry analysis (PAHs, persistent organochlorines) for blood will be conducted by the NOAA/NMFS Northwest Fisheries Science Center, which is also the designated laboratory for the chemical analysis of remote biopsies and analyses of stranded animal tissues. NWFSC has been a part of the NIST interlaboratory comparison program for over 15 years.

Urine samples contained in glass containers will also be analyzed for PAH metabolites, including glucuronide metabolites, using high-performance liquid chromatography/fluorescence detection (HPLC/uvf) at the NOAA/NMFS Northwest Fisheries Science Center (NWFSC).

Chromatograms will be recorded at three fluorescence wavelength pairs where the parent compounds and metabolites fluoresce: 2-3 ring compounds such as naphthalene at 293/335 nm, 3-4 ring compounds such as phenanthrene at 260/380 nm, and 4-5 ring compounds such as benzo(a)pyrene at 380/430 nm. These three wavelength pairs were chosen because the parent

compounds, naphthalene, phenanthrene and benzo(a)pyrene are often found in contaminated marine sediments. Naphthalene, phenanthrene, naphthalene-related and phenanthrene-related compounds come from petrogenic sources (e.g., diesel fuel or crude oil). Benzo(a)pyrene like compounds come from pyrogenic sources (e.g. combustion of fossil fuels).

Acquisitions / Grants / Contracts:

It is anticipated that the funding for CZS and LDWF can be handled directly through a contract with the Responsible Party or NOAA. Other contractor funding will be handled through the PIs' existing NOAA contracts.

Data Handling and Sharing:

NRDA chain-of-custody procedures will be observed at all times for samples designated to contribute to the MC 252 NRDA. In addition to raw data, results of laboratory analyses and metadata from sample collection will be integrated into an Access database and uploaded to the NOAA NRDA site in a timely fashion and made available to BP and/or its' representatives.

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 BP (or 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 BP (or ENTRIX on behalf of BP). 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 simultaneously to all trustees and BP (or ENTRIX on behalf of BP). 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. In order to assure reliability of the consensus data and full review by the parties, no party shall publish consensus data until 7 days after such data has been made available to the parties. Also, 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 to BP (or ENTRIX on behalf of BP).

Table 1. Health parameters to be measured from dolphin blood & urine samples

Hematology (Cornell)	Electrophoresis (Cornell)	Serum chemistry (Cornell)	Endocrinology (Cornell)	Functional Immunology (UConn)	Urinalysis (on vessel)
hematocrit	total protein	electrolytes: sodium, potassium, chloride	total thyroxine	T-lymphocyte proliferation	color
packed cell volume	albumin	bicarbonate	free thyroxine	B-lymphocyte proliferation	turbidity
hemoglobin	alpha 1, alpha 2 & total alpha globulin	anion gap	total triiodothyronine	neutrophil phagocytosis	pH
red blood cell count	beta 1, beta 2 & total beta globulin	urea nitrogen	progesterone	monocyte phagocytosis	specific gravity
MCV, MCH, MCHC	gamma globulin	creatinine	estradiol		glucose
red cell distribution width		uric acid	aldosterone		bilirubin
reticulocyte count		calcium, phosphate, magnesium	testosterone		ketone
white blood cell (WBC) count & differential: segmented neutrophils, band neutrophils, lymphocytes, monocytes, eosinophils		total protein, albumin, globulin	cortisol		blood
platelet count		glucose			protein
plasma appearance		enzymes: ALT, AST, SDH, LDH, AP, GGT			urobilinogen
RBC morphology, WBC exam, parasites		total, direct, & indirect bilirubin			nitrite
		amylase			leukocytes
		cholesterol, triglycerides			
		creatinine kinase			
		iron, total iron binding capacity, saturation			
		lipemia, hemolysis, icterus			

Table 2. Chemical contaminants to be measured in dolphin blood samples. Chemical contaminant analyses will be conducted by NOAA/NMFS.

Σ Low molecular weight PAHs	Σ High molecular weight PAHs	Σ PCBs	Σ DDT's	Σ Chlordanes	Σ HCHs	Σ PBDEs
naphthalene C1-throughC4-naphthalenes acenaphthylene acenaphthene fluorene C1-throughC3-fluorenes phenanthrene C1-throughC4-phenanthrenes/anthracenes dibenzothiophene C1-throughC4-dibenzothiophenes anthracene	fluoranthene pyrene C1-throughC4-fluoranthenes/pyrenes benz[a]anthracene chrysene/triphenylene C1-throughC4-chrysenes/benz[a]anthracenes benzo[b]fluoranthene benzo[j]fluoranthene/benzo[k]fluoranthene benzo[e]pyrene benzo[a]pyrene perylene indeno[1,2,3-cd]pyrene dibenz[a,h]anthracene benzo[ghi]perylene	Congeners: 17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 82, 87, 95, 99, 101/90, 105, 110, 118, 128, 138/163/164, 149, 151, 153/132, 156, 158, 170, 171, 177, 180, 183, 187/159/182, 191, 194, 195, 199, 205, 206, 208, 209	<i>o,p'</i> - DDD <i>p,p'</i> - DDD <i>o,p'</i> - DDE <i>p,p'</i> - DDE <i>o,p'</i> - DDT <i>p,p'</i> - DDT	Oxychlordane <i>gamma</i> -chlordane nona-III-chlordane <i>alpha</i> -chlordane <i>trans</i> -nonachlor and <i>cis</i> -nonachlor	<i>alpha</i> -, <i>beta</i> -, and <i>gamma</i> - HCH isomers	Congeners: 28, 47, 49, 66, 85, 99, 100, 153, 154, 183

YEAR



Figure 1. Locations of dolphin sightings from mark-recapture abundance surveys conducted between June and November, 2010.

COST ESTIMATE
FISCAL YEAR (FY) 2011

ITEM	RATE	CALCULATION BASIS	BUDGET
Invitational Travel Daily			\$55,720
Invitational Travel Transportation			\$37,247
TOTAL TRAVEL			\$92,967
Field Operations			
Transport & maintenance for 6 Capture Vessels			\$7,567
Boat Fuel/Oil for Capture Vessels			\$10,810
TOTAL FIELD OPERATIONS			\$18,377
Supplies/Equipment/Shipping			
Blood and sample collection supplies			\$12,000
Medical/safety supplies/drugs			\$6,000
Blood Shipping	\$50	per day	\$750
Misc. Supplies, Duplicating, etc.		Batteries, pipettes, etc.	\$2,000
Dolphin handling, tagging, measuring gear			\$1,350
Misc laboratory supplies		formalin, vials, slides microtome blades, etc.	\$2,000
TOTAL SUPPLIES			\$102,100
FTE Salaries		one month (prep, field-work, breakdown) * 15 FTEs	\$121,613
Contracts			
Blood chemistry, hematology, endocrinology, urinalysis	\$250	per dolphin	\$12,500
Immunology	\$1,000	per dolphin	\$50,000
Safety Support, Paramedic	\$750	daily rate	\$7,500
PAH analysis blood	\$990	per dolphin	\$49,500
PAH metabolites in urine	\$283	per dolphin	\$14,150
TOTAL CONTRACTS			\$133,650
Labor Contracts			
Contract Labor			\$125,720
OT for contract staff			\$30,000
TOTAL CONTRACT LABOR			\$155,720
TOTAL COSTS NOAA			\$546,426

ITEM	RATE		BUDGET
Chicago Zoological Society Contract			
Field personnel/salaries			\$15,000
Field operations			\$8,000
SubContracts (catcher, veterinarian)			\$35,500
Total Direct Costs			\$58,500
Indirect Costs (2011 federally negotiated rate)			\$41,868
VHF Tags	\$250	per dolphin, only tag Barataria dolphins	\$7,500
Satellite Tags	\$1,900	per dolphin, only tag Barataria dolphins	\$57,000
TOTAL COSTS CZS			\$164,868

Louisiana Department of Wildlife & Fisheries			BUDGET
Fuel			\$10,000
Tracking equipment & camera			\$13,000
Labor			\$44,805
Vehicle/vessel cost			\$10,000
TOTAL COSTS LDWF			\$77,805

TOTAL COSTS: \$789,099

The Parties acknowledge that this budget is an estimate, and that actual costs may prove to be higher. BP's commitment to fund the costs of this work includes any additional reasonable costs within the scope of this approved work plan that may arise. The trustees will make a good faith effort to notify BP in advance of any such increased costs.

Durable Equipment - All durable equipment (such as cameras, GPS, etc.) purchased by BP for this study will be returned to BP or their designated representatives at the conclusion of its use for this study. Radio tags that are recovered or are not deployed will be returned to BP or its designated contractor at the end of this study, unless otherwise agreed.

References

1. Bodkin JL, Ballachey BE, Dean TA, Fukuyama AK, Jewett SC, McDonald L, Monson DH, O'Clair CE, VanBlaricom GR: **Sea otter population status and the process of recovery from the 1989 'Exxon Valdez' oil spill.** *Mar Ecol-Prog Ser* 2002, **241**:237-253.
2. Golet GH, Seiser PE, McGuire AD, Roby DD, Fischer JB, Kuletz KJ, Irons DB, Dean TA, Jewett SC, Newman SH: **Long-term direct and indirect effects of the 'Exxon Valdez' oil spill on pigeon guillemots in Prince William Sound, Alaska.** *Mar Ecol-Prog Ser* 2002, **241**:287-304.
3. Matkin CO, Saulifis EL, Ellis GM, Olesiuk P, Rice SD: **Ongoing population-level impacts on killer whales *Orcinus orca* following the 'Exxon Valdez' oil spill in Prince William Sound, Alaska.** *Mar Ecol-Prog Ser* 2008, **356**:269-281.
4. Hansen L, Wells R: **Bottlenose dolphin health assessment: Field report on sampling near Beaufort, North Carolina, during July, 1995.** In: Miami, Florida: NOAA Fisheries; 1996: 24.
5. Schwacke LH, Twiner MJ, De Guise S, Balmer BC, Wells RS, Townsend FI, Rotstein DC, Varela RA, Hansen LJ, Zolman ES *et al*: **Eosinophilia and biotoxin exposure in bottlenose dolphins (*Tursiops truncatus*) from a coastal area impacted by repeated mortality events.** *Environ Res* 2010, **110**(6):548-555.
6. Scott M, Wells R, Irvine A: **A long-term study of bottlenose dolphins on the west coast of Florida.** In: *The bottlenose dolphin*. Edited by Leatherwood S, Reeves RR. San Diego, CA, USA: Academic Press; 1990: 235-244.
7. Wells R, Rhinehart H, Sweeney J, Townsend F, Casper D, Hansen L: **Assessment of health of bottlenose dolphin populations in Sarasota Bay, Florida and Matagorda Bay, Texas.** In: *11th Biennial Conference on the Biology of Marine Mammals: 1995; Orlando, Florida*; 1995.
8. Wells RS, Rhinehart HL, Hansen LJ, Sweeney JC, Townsend FI, Stone R, Casper D, Scott MD, Hohn AA, Rowles TK: **Bottlenose dolphins as marine ecosystem sentinels: Developing a health monitoring system.** *EcoHealth* 2004, **1**:246-254.
9. Schwacke L, Hall A, Townsend F, Wells R, Hansen L, Hohn A, Bossart G, Fair P, Rowles T: **Hematologic and serum biochemical reference intervals for free-ranging common bottlenose dolphins (*Tursiops truncatus*) and variation in the distributions of clinicopathologic values related to geographic sampling site.** *Am J Vet Res* 2009, **70**(8):973-985.
10. Schwacke L, Zolman E, Balmer B, Guise SD, Hoguet J, Hohn A, Kucklick J, Lamb S, Levin M, Litz J *et al*: **Anemia, hypothyroidism and immune suppression associated with PCB exposure in bottlenose dolphins (*Tursiops truncatus*) from the Coast of Georgia, USA.** *Environ Health Perspect* Submitted.
11. Schwacke LH, Twiner MJ, De Guise S, Balmer BC, Wells RS, Townsend FI, Rotstein DC, Varela RA, Hansen LJ, Zolman ES *et al*: **Eosinophilia and biotoxin exposure in bottlenose dolphins (*Tursiops truncatus*) from a coastal area impacted by repeated mortality events.** *Environ Res* 2010, **110**(6):548-555.
12. Schwacke LH: **Off the charts: polychlorinated biphenyl levels in coastal dolphins and insights into human exposure.** In: *Annual Meeting of the American Association for the Advancement of Science*. San Diego, California; 2010.
13. Loughlin T, Cunningham L, Gales N, Wells R, Boyd I: **Marking and capturing.** In: *Marine Mammal Ecology and Conservation: A Handbook of Techniques*. Edited by Boyd I, Bowen D, Iverson S: Oxford University Press; 2010: 16-41.

14. Wells R, Borrell A, Aguilar A, Rowles T, Rhinchart H, Hofmann S, Jarman W, Hohn A, Sweeney J: **Integrating life history and reproductive success data to examine potential relationships with organochlorine compounds for bottlenose dolphins (*Tursiops truncatus*) in Sarasota Bay, Florida.** In.: International Whaling Commission; 2004.
15. Wells R: **Identification methods.** In: *Encyclopedia of Marine Mammals*. Edited by Perrin W, Thewissen BWJ, 2nd edn. San Diego, CA: Elsevier, Inc; 2009.
16. **Marine Mammal Protection Act.** In: *16 USC Chapter 31*. vol. 16 USC Chapter31; MMPA 1972.
17. DeGuise S, Bernier J, Dufresne M, Martineau D, Beland P, Fournier M: **Immune functions in beluga whales (*Delphinapterus leucas*): evaluation of mitogen-induced blastic transformation of lymphocytes from peripheral blood, spleen and thymus.** *Veterinary Immunology and Immunopathology* 1996, **50**:117-126.
18. DeGuise S, Flipo D, Boehm J, Martineau D, Beland P, Fournier M: **Immune functions in beluga whales (*Delphinapterus leucas*): evaluation of phagocytosis and respiratory burst with peripheral blood leukocytes using flow cytometry.** *Veterinary Immunology and Immunopathology* 1995, **47**:351-362.
19. Mori C, Morsey B, Levin M, Gorton TS, De Guise S: **Effects of organochlorines, individually and in mixtures, on B-cell proliferation in marine mammals and mice.** *J Toxicol Env Heal A* 2008, **71**(4):266-275.
20. Levin M, Leibrecht H, Mori C, Jessup D, De Guise S: **Immunomodulatory effects of organochlorine mixtures upon in vitro exposure of peripheral blood leukocytes differ between free-ranging and captive southern sea otters (*Enhydra lutris*).** *Veterinary Immunology and Immunopathology* 2007, **119**(3-4):269-277.
21. De Guise S, Levin MJ, Mori C, Morsey B, Nambiar P: **Immunotoxicity of PCBs in marine mammals, mouse and humans upon in vitro exposure.** *Mar Environ Res* 2006, **62**:S370-S371.
22. Levin M, Morsey B, Mori C, De Guise S: **Specific non-coplanar PCB-mediated modulation of bottlenose dolphin and beluga whale phagocytosis upon in vitro exposure.** *J Toxicol Env Heal A* 2004, **67**(19):1517-1535.
23. McFee WE, Schwacke JH, Stolen MK, Mullin KD, Schwacke LH: **Investigation of growth phases for bottlenose dolphins using a Bayesian modeling approach.** *Mar Mamm Sci* 2010, **26**(1):67-85.
24. Hohn A, Scott M, Wells R, Sweeney J, Irvine A: **Growth layers in teeth from free-ranging, known-age bottlenose dolphins.** *Mar Mamm Sci* 1989, **5**(4):315-342.
25. Varanasi U, Stein J, Nishimoto M: **Biotransformation and disposition of polycyclic aromatic hydrocarbons (PAH) in fish.** . In: *Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment*. Edited by Varanasi U. Boca Raton, FL: CRC Press; 1989: 94-149.