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NATURAL RESOURCE DAMAGE ASSESSMENT
WORK PLAN FOR DETERMINING PHYSIOLOGICAL INJURY
TO OILED BIRDS FROM THE
DEEPWATER HORIZON (MC 252) OIL SPILL

Prepared by:

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INTRODUCTION

The Deepwater Horizon (MC 252) oil spill began April 22, 2010. The Natural Resource Trustees for this oil spill that have particular interest in birds include, but are not limited to, the U.S. Fish and Wildlife Service (Service), the National Park Service, and the natural resource agencies of the States of Texas, Louisiana, Mississippi, Alabama, and Florida. The Trustees are authorized under the Oil Pollution Act (33 U.S.C. 2701 *et seq.*) and the Comprehensive Environmental Response, Compensation and Liability Act (42 U.S.C. 9601 *et seq.*) to assess natural resource damages associated with the harm caused to natural resources by the releases of hazardous substances and discharges of oil. The activities proposed in this study plan are part of the natural resource damage assessment (NRDA) being conducted by the Trustees.

Oil spill related injury to wildlife is of major concern to the natural resource trustees, BP and the American public. Seabirds, colonial waterbirds, coastal marsh birds, and shorebirds are susceptible to impacts from the oil. Several work plans have been developed to concurrently evaluate oil spill related injuries to these different avian guilds. This plan specifically seeks to address injury to oiled birds by collecting post-oil spill exposure physiological data from birds admitted to rehabilitation centers in the Gulf. This study plan contemplates avian injury assessment work through May 2011 for budget purposes only. It may be necessary to extend the time frame and scope of this work plan to further assess long-term oil-related impacts to birds in the region.

Physiological Disruptions: The Mechanistic Link between Oil Exposure and Avian Mortality

An evaluation of physiology is an important part of establishing injury and will provide critical information on oil-induced adverse impacts. It is also critically important to evaluate these physiological indicators of oil exposure to strengthen the connection between oil exposure and death of birds. Currently, the cause of death of oiled birds remains an important unknown, although hemolytic anemia is a likely contributing mechanism in many cases. Hemolytic anemia causes reduced availability of oxygen to tissues which consequently leads to anaerobic metabolism, altered cell membrane permeability, cellular and tissue dysfunction, and ultimately organ failure. In a controlled dosing study in Atlantic Puffins (*Fratercula arctica*) and Herring Gulls (*Larus argentatus*), Leighton et al. (1983) demonstrated a dramatic reduction in the number of circulating red blood cells in birds orally exposed to crude oil. The presence of damaged hemoglobin in these red blood cells, as evidenced by Heinz body inclusions, points to oxidative damage as a mechanism of anemia. Troisi et al. went further to demonstrate a strong correlation between Heinz bodies, and the plasma proteins haptoglobin and ferritin, which indicate red blood cell destruction and oxidative damage, and circulating PAH concentration in plasma in oiled Common Guillemots (*Uria aalge*) (2007). While the pathophysiology following exposure to crude oil may be multifactorial, oxidative hemolytic anemia likely plays a key mechanistic role in morbidity and mortality. If this is the case, then character and severity of anemia, presence of Heinz bodies, and changes in haptoglobin and ferritin may be predictive of survival in oiled birds.

OBJECTIVE

The objective of this study is to determine whether hemolytic anemia is a key diagnostic feature in birds oiled by the Deepwater Horizon (MC 252) oil spill and whether biomarkers of hemolytic anemia are consistently related to mortality in severely oiled birds.

Study Components:

- Evaluate physiological biomarkers of hemolytic anemia in oiled birds admitted to rehabilitation centers in the Gulf
- Relate physiological biomarkers of oil exposure to circulating levels of PAHs and the degree of external oiling observed on birds
- Determine whether hemolytic anemia is a probable contributing mechanism to the death of severely oiled birds

Coordination with other Deepwater Horizon (MC252) studies

This project is not being conducted in collaboration with B.P.

STUDY DESIGN

This study will rely on sampling birds opportunistically from rehabilitation centers in the Gulf of Mexico. Blood sampling will be done safely, non-lethally, and with minimal distress to the sampled birds. When the proper precautions are taken, blood collection from birds is a safe technique approved by the American Ornithologists Union (1988) that does not adversely affect territorial behaviors, reproductive parameters, or annual returns rates/survival (Hosyak and Weatherhead, 1991). We will target species that are frequently admitted to rehabilitation centers (e.g., gulls and pelicans) but may opportunistically sample other species when they are admitted. Briefly, all blood samples will be collected from brachial or medial metatarsal veins using sterile 26 G needles and 3 mL syringes and 70 μl capillary tubes. Total volume of blood from each individual will be less than 1% of body mass. Copies of all medical and field records associated with each sampled bird will be archived at Virginia Tech.

We will collect blood from 30 oiled birds of at least two species from August-December 2010. Upon admission, we will attempt to collect blood from individuals of each species across a wide range of externally visible oiling (from lightly oiled releasable birds, to severely oiled birds that eventually die). Because we are specifically concerned with establishing hemolytic anemia as a contributing cause of death in oiled birds, we will sample as many dying (e.g., to be euthanized upon admission to the clinic) birds as possible. Desired sample sizes are approximate due to lack of measures of variance that are representative of what we may find in this system. After we have initial samples and analyses we will re-examine the sample size needed.

Task 1. Conduct a physical exam and collect blood samples from oiled birds admitted to rehabilitation centers for determination of extent of oil exposure.

Among the birds admitted to rehabilitation centers, it is critical to assess their relative degree of exposure in order to determine relationships between exposure, adverse physiological responses, and mortality. We will use two methods to quantify the relative degree of exposure to oil. First, we will utilize the index provided by the USFWS (Oiled Bird Observation Form) to designate birds as not visibly oiled, or as having trace, light, moderate, or heavy oiling. Second, we will analyze blood for PAHs in larger species (e.g., gulls and pelicans), providing clear quantification of circulating toxicant concentrations in oiled birds. We will compare these indices of exposure to determine how the extent of external oiling relates to internal circulating levels of toxicants. We will then mathematically relate these two indices of exposure to adverse effects (i.e., see Task 2 for physiological disruptions and probability of mortality).

***NOTE**: Funding for PAH analyses of birds is beyond this scope of work and will need to be provided by DOI in order to complete the project.*

Task 2. Measure physiological biomarkers in oiled birds admitted to rehabilitation centers to determine whether hemolytic anemia is a consistent symptom of oiling in birds and whether it is a potential contributing cause of death in severely oiled individuals.

A suite of well established physiological indicators of hemolytic anemia will be used to determine whether birds admitted to rehabilitation centers manifest physiological impairment that can be linked to exposure to polycyclic aromatic hydrocarbons (PAHs). These metrics of physiological response are significant to organismal health, and are established symptoms of toxicity in birds exposed to crude oil and/or PAHs (Leighton et al., 1983; Troisi and Borjesson, 2005; Troisi et al., 2006, 2007). To assess whether exposure to oil has caused symptoms of hemolytic anemia, we will quantify hematocrit, total hemoglobin, mean corpuscular hemoglobin concentration, and the plasma proteins, ferritin and haptoglobin, indicative of oxidative damage to red blood cells. In addition, we will cytologically evaluate and quantify Heinz bodies in red blood cells and reticulocytes via light microscopy. Heinz bodies (Heinz-Ehrlich bodies) are inclusions in red blood cells resulting from oxidative injury to and precipitation of hemoglobin and are associated with exposure to PAHs (Leighton et al., 1983; Troisi & Borjesson 2005,Troisi et al., 2006, 2007). Reticulocytes are immature red blood cells which increase in circulation in response to anemia. Reticulocytes will be expressed as a percentage of red blood cells. Hematocrit will be expressed as the percent of blood volume comprised of packed cells, as determined from centrifugation. Hemoglobin is the oxygen carrying protein of vertebrate red blood cells, and will be quantified via reflectance photometry and reported in g/dl. Mean corpuscular hemoglobin concentration is a value calculated from hematocrit and hemoglobin and helps to further define anemic conditions. For ferritin and haptoglobin, plasma samples can be collected and stored at -80 °C until assayed in the laboratory. Both proteins will be quantified and expressed in nanogram/milliliter using calorimetric assays described in Troisi et al., 2007. A total of 500 microliters of plasma, flash frozen in liquid nitrogen, will be required from each individual.

BUDGET NARRITIVE

Staff: The principle investigator (PI) salary is not included in this initial budget, but will need to be covered in future budgets if the project is continued/expanded. The PI is responsible for overall scientific, fiscal and personnel management on the project. He is the principle liaison with involved NRDA partners. He will directly supervise any graduate students, technicians, and research scientists involved with the project and ensure that the direction of the project is consistent with this SOW and the needs of the NRDA. The co-PI (Dr. Jesse Fallon) is the Ph.D. student who will be responsible for training staff to collect and store blood samples and to prepare slides for cytological analyses. He will also be responsible for all microscopy and conducting assays in the laboratory. An hourly wage technician will be used to assist with blood sampling and for sample processing in the laboratory.

Equipment:

Dewars flask: This flask is needed to flash freeze samples on liquid nitrogen and store them at the rehabilitation center until they can be transferred to VA Tech.

Olympus recording microscope with camera: One of the key assays in this proposal is the count of Heinz bodies and reticulocytes and photographic documentation of our findings.

Centrifuge: Blood assays require that blood be centrifuged after collection. Thus a small portable centrifuge will be needed at the rehabilitation centers.

BUDGET (*Note: PAH analyses not included*)

	Start Date: August 1, 2010
Budget Items	$$
Bill Hopkins	$0
Jesse Fallon, GRA PhD Step 18	$25,110
Technician (Wage, 6 months, $10/hr)	$9,600
Fringe Benefits (GRA and Tech @ 6.75%)	$2,705
Tuition	$9,399
Equipment (centrifuge 2.5K, microscope/imaging 7K, Dewars flask 2K)	$11,500
Materials/Supplies/Reagents	$14,000
Travel	$11,000
TOTAL DIRECT COSTS	$83,314
INDIRECT COSTS (26%, tuition and equipment exempt)	$16,228
TOTAL COSTS	**$99,542**

LITERATURE CITED

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

APPROVAL

Trustee NRDA Bird Group Lead	8/2/10 Date
State of Louisiana Trustee Representative	6/27/15 Date