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EVALUATING BLOOD PARAMETERS AS A MEASURE OF PHYSIOLOGICAL INJURY TO OILED BIRDS FROM THE DEEPWARER HORIZON (MC252) OIL SPILL

DRAFT REPORT

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EXECUTIVE SUMMARY

The Deepwater Horizon oil spill in 2010 released an unprecedented volume of crude oil into the Gulf of Mexico. While avian mortality associated with this and other oil spills has been documented, the sublethal physiological effects of oil exposure on birds with moderate and light oiling is not yet clearly understood. In this study, we found that American oystercatchers, black skimmers, brown pelicans, and great egrets with small amounts of visible oil present on their feathers can suffer from oxidative injury to erythrocytes (i.e., red blood cells), have decreased numbers of circulating erythrocytes, and have evidence of a regenerative hematological response in the form of increased reticulocytes (i.e., young red blood cells) (Summary Table 1). Specifically, we found Heinz bodies present almost exclusively in birds from sites impacted with oil – a change pathognomonic for oxidative injury. Further, we found that packed cell volume (PCV) values were 4% to 19% lower in birds with visible oil than birds from reference sites and that there were 27% to 40% more reticulocytes present in birds with visible oil than birds from reference sites. All of these responses are consistent with hemolytic anemia caused by oil exposure. Of the four primary species examined, brown pelicans and great egrets had the most consistent evidence of adverse effects. Together these findings provide evidence that relatively modest oil exposure, based on visible oiling of feathers, can cause hemolytic anemia. Furthermore, we found that birds with no visible oiling captured from the area potentially impacted by the spill also had Heinz body formation, increased numbers of reticulocytes, and reduced packed cell volumes when compared to birds from reference sites. This observation suggests that even birds without visible oil suffered physiological injury from the spill, with ingestion of oil or oil-related contaminants during foraging or preening as a likely additional

route of exposure. Anemia causes a decrease in oxygen availability to tissues, and can induce muscle fatigue, lethargy, decreased energy availability for metabolic processes, adverse reproductive impacts, and may have implications for survival and fitness. In addition to the hematological effects we found in birds from areas of potential impact, our results also indicate that visible oiling is a better measure of potential physiological impact than circulating PAH levels, suggesting that visible oil evaluation should be a key component of damage assessment efforts. Furthermore, we found that fluorescence under ultraviolet light assessment (UV oiling) identified many birds with small amounts of oil exposure that appeared to be oil-free on visual exam, supporting its utility as a simple tool for improving detection of oiled birds in the field. The relationships between UV-detectable oil and physiological injury were similar to those made with visual oil assessment alone and provided insight into the occurrence of injury in birds that had no visible oiling.

INTRODUCTION

Assessing the impact of oil spills on wildlife is of concern to industries, governments, and the general public (Norse and Amos 2010). Historically, mortality estimates have served as the basis for assessing impact to natural resources, as well as for directing resources toward mitigation and restoration efforts. Acute avian mortality has been described for many large-scale oil spills, such as the Exxon Valdez (e.g. Piatt et al. 1990, Iverson and Esler 2010), Prestige (e.g. Munilla et al. 2011), and more recently, Deepwater Horizon (USFWS 2011). These acute mortality data are important; however, they likely underestimate the more widespread impact of oil exposure on individuals, as well as the long-term consequences on populations and ecosystems (Velando et al. 2005; Votier et al. 2005, Iverson and Esler 2010, Henkel et al. 2012). Prolonged adverse effects from oil spills have been identified, including delayed onset of population declines and slow population recovery (Peterson et al. 2003), but the effects of modest oil exposure on individuals have yet to be identified.

While acute avian mortality is well documented, the sublethal physiologic impact of crude oil on wild birds has not been thoroughly investigated, and the mechanistic cause of lethality remains unclear. Myriad negative effects of the polycyclic aromatic hydrocarbons (PAHs) found in crude oil on marine, aquatic, and terrestrial ecosystems have been described (Peakall et al. 1982). Insult from oil exposure spans an array of physiologic effects, including inflammation, immunosuppression, and oxidative damage to cells (Fry et al. 1986, Leighton 1995, Briggs et al. 1996, Golet et al. 2002). These sublethal effects can negatively impact growth, alter organ function, reduce reproductive success, and likely increase risk of disease (Briggs et al. 1996, Esler et al. 2000, Giese et al. 2000, Eppley and Rubega 1990, Alonso-

Alvarez et al. 2007). These factors can coalesce to reduce fitness in individuals and negatively impact populations on a much larger scale than the results of acute mortality events alone (Esler et al. 2002, Golet et al. 2002). Uncovering the mechanisms behind and the outcomes of this widespread distribution of sublethal, modest oil exposure are critical to a more comprehensive understanding of the impact of oil spill events. Of the sublethal, physiological impacts resulting from avian exposure to oil spills, oxidative damage to erythrocytes and subsequent anemia are of interest to oil spill investigators, as such injury can be evaluated in blood samples taken from live birds.

Anemia, defined as a reduction in circulating erythrocytes and/or of the oxygen-carrying bioprotein hemoglobin (Hb), causes reduced availability of oxygen to tissues, which consequently leads to anaerobic metabolism, altered cell membrane permeability, cellular and tissue dysfunction, and if it progresses, ultimately organ failure (Greenburg 1996, Fried 2009). These physiological manifestations of anemia have obvious implications for survival and fitness, because even mild anemia can cause muscle fatigue, lethargy, and reduced energy availability for cellular metabolism (Latimer et al. 2003). Among their varied physiological insults, exposure to PAHs results in oxidative injury to cell membranes and cytoplasmic Hb (Latimer et al. 2003). When exposed to reactive oxygen species (ROS), iron within the Hb molecule undergoes a change in charge, which results in protein denaturation. Aggregates of this damaged Hb can be highlighted by certain cell staining techniques (e.g., new methylene blue vital staining), resulting in readily identifiable inclusion bodies within erythrocytes (Latimer et al. 2003). These aggregations of denatured Hb, called Heinz body inclusions (also, Heinz-Ehrlich bodies) are pathognomonic for oxidative damage to erythrocytes. These damaged, abnormal erythrocytes

undergo lysis or are destroyed *in vivo*, a process known as hemolysis. Heinz body hemolytic anemia induces fatigue and a reduction in energy availability for metabolic processes (Butler et al. 1986).

Hemolytic anemia has been demonstrated in several species of birds exposed to crude oil (Leighton et al. 1985, Fry and Lowenstine 1985, Leighton 1986, Yamato et al. 1996, Troisi et al. 2007). It is believed that oxidative damage is mediated by metabolites of PAHs generated from metabolic actions of cytochrome P450 enzymes (Troisi et al. 2006, 2007). In controlled dosing studies of Atlantic Puffins (*Fratercula arctica*) and Herring Gulls (*Larus argentatus*), Leighton et al. (1985, 1986) demonstrated a dramatic reduction in the number of circulating RBCs in birds orally dosed with large volumes of crude oil. Further, the presence of damaged Hb in the erythrocytes from these birds, as evidenced by Heinz body inclusions, identifies oxidative damage as a mechanism of anemia (Leighton et al. 1983, 1985, 1986). In birds admitted to rehabilitation facilities, Troisi et al. (2007) went further by demonstrating a correlation between the number of Heinz bodies and circulating PAH concentrations in plasma from heavily-oiled Common Guillemots (*Uria aalge*), suggesting a dose-response relationship. While these studies provide evidence of a correlation between oil exposure, PAH levels in the blood, and Heinz bodies in Guillemots, Puffins, and Herring Gulls, the degree of hematologic effects of oil is likely species-specific, prompting a need for evaluation of birds in other families. Further, these studies relied on heavy oiling and large doses in experimental exposures, and there is a paucity of data on birds with relatively modest oiling, which may represent a more common occurrence in most spill events.

In this study we examined the relationships between oiling and physiological parameters, including a suite of hematological values, in several avian species following the Deepwater Horizon oil spill. We evaluated relationships in birds between visible oiling, ultraviolet fluorescence (UV oiling), circulating PAH levels, and hematologic parameters, biochemical enzyme and electrolyte profiles, ferritin and haptoglobin. We focused on American oystercatchers (*Haematopus palliatus*, AMOY), black skimmers (*Rynchops niger*, BLSK), brown pelicans (*Pelecanus occidentalis,* BRPE), clapper rails (*Rallus longirostris*, CLRA) great egrets (*Ardea alba,* GREG), and seaside sparrows (*Ammodramus maritimus*, SESP) from impacted and reference sites. Unlike other studies that have predominately focused on heavily oiled birds (e.g., Leighton et al. 1985, Yamato et al. 1996, Troisi et al. 2007), the approach used here provides a comprehensive assessment of the hematological and biochemical effects of trace and light amounts of crude oil on these species.

Avian Responses to Oil Exposure

Heinz bodies are indicators of oxidative injury to erythrocytes (Latimer et al. 2003). These intracytoplasmic, round inclusions can be highlighted with supravital staining techniques, such as new methylene blue. These staining techniques must be performed on live cells for proper stain uptake (Latimer et al. 2003; Fallon et al. 2013).

Circulating erythrocytes can be quantified in circulation in two ways. The first quantification method is packed cell volume (PCV), which is the percentage of whole blood volume that is erythrocytes. The second approach is the red blood cell count (RBC) which is a value that is calculated by manual or automated methods and is reported as the number of

erythrocytes per volume of blood. Both PCV and RBC typically change in a similar manner, although their values can be altered by cell size or mean cell volume (MCV) (Campbell and Ellis 2007).

In vertebrates, the appropriate physiological response to anemia is the release of younger blood cells, termed reticulocytes because of their meshwork of retained RNA, from hematopoietic organs (Goodnough et al. 2000, Campbell and Ellis 2007). A decrease in circulating oxygen, due to anemia, triggers the release of reticulocytes into the bloodstream, a process designated as a regenerative response (Figure 1) (Rosse and Waldmann 1962). These cells tend to be larger than mature erythrocytes, which leads to an increase in MCV. In cases of hemolytic anemia, the mean corpuscular Hb concentration (MCHC), or the average hemoglobin per cell, does not change from normal concentration or may be artifactually increased due to free Hb in the bloodstream.

In addition to these basic hematologic changes following exposure to crude oil, there is evidence that concentrations of the plasma proteins haptoglobin and ferritin are correlated with oxidative hemolysis (Troisi et al. 2007). While Hb is critical to oxygen transport within erythrocytes, it can have negative effects on tissues if it is free within circulation (Chamanza et al. 1999). Haptoglobin, an acute-phase glycoprotein, functions primarily to bind free Hb and escort it safely to the liver, thereby serving to protect cells and to recycle iron (Chamanza et al. 1999). Haptoglobin is initially up-regulated in response to Hb leakage; however, in cases of hemolytic anemia when there are excessive amounts of free Hb, haptoglobin reserves may be depleted, resulting in low plasma levels (Chamanza et al. 1999, Trevisan et al. 2001). In birds,

this has been demonstrated both experimentally (Prichard et al. 1997) and via environmental exposure to crude oil (Troisi et al. 2007).

Ferritin (FT) is a protein that functions to store iron in a bioavailable form (Seiler 1978). During acute inflammatory responses, plasma FT increases to protect against Fe (III)-oxidation of bio-molecules (Darcel and Merriman 1971). Circulating levels of FT can function clinically as an indirect measure of total body iron concentration (Cook et al. 2003). Ferritin has been demonstrated to increase in cases of PAH-induced hemolytic anemia in oiled Guillemots (Troisi et al. 2007).

In addition to hematological injury, exposure to crude oil has the potential to induce changes in enzymes and electrolytes (e.g., Prichard et al. 1997, Alonso-Alvarez et al. 2007). These clinical biochemistry parameters can help identify changes associated with insufficient renal function, hepatic insult, and electrolyte imbalance. These values are also useful because they are routinely quantified in clinical settings, and reported reference ranges exist for a number of species including AMOY (Carlson-Bremer et al. 2010), BRPE (Zaias et al. 2000), and GREG (Hoffman et al. 2005).

METHODS

Study area and focal species

Field sampling and bird captures were conducted under three DWH NRDA pre-assessment avian capture surveys (Work Plan for Estimating Secretive Marsh Bird Mortality, Deepwater Horizon Oil Spill, NRDA Bird Study #3, June 7, 2010; Work Plan for Estimating Oiling and Mortality of Colonial Waterbirds from the Deepwater Horizon Oil Spill, NRDA Bird

Study #4, July 10, 2010; Work Plan for Estimating Shorebird Oiling and Mortality Deepwater Horizon Mississippi Canyon 252 Oil Spill, August 13, 2010; details at http://www.doi.gov/deepwaterhorizon/index.cfm). Field collaborators/personnel captured AMOY, BLSK, BRPE, CLRA, GREG, and SESP from reference areas and areas of potential impact (hereafter API) from June 20th, 2010 until February 23rd, 2011 (Table 1, Figures 2 and 3).

Our six focal species represent a diversity of ecological niches which could influence their relative susceptibility to oil exposure. Clapper rails and SESP were selected for this study because of their preferred habitat and relatively small home range during the summer months, which coincides with the aftermath of the Deepwater Horizon spill (Post and Greenlaw 2009; Rush et al. 2012). These secretive marsh birds are year-round residents along the Gulf Coast, and inhabit salt marshes surrounded by open water – sites that were commonly impacted by oil from the Deepwater Horizon spill. Clapper rails and SESP are both omnivorous, eating both seeds and marine invertebrates (Post and Greenlaw 2009; Rush et al. 2012). American oystercatchers are unique among the other species evaluated here because they feed almost exclusively on marine invertebrates. Black skimmers, which are recognized as a species with declining populations, were selected because of their unique surface water foraging strategy and because their nesting habits (sand and shell beaches and islands) put them at high risk for exposure to oil (Gochfeld and Burger 1994). Brown pelicans eat mostly small fish but are at high risk of dermal exposure to oil because they capture their food most often by diving head first after prey (Shields 2002). Great egrets forage for food in a wide range of habitats and are unique among the other species evaluated here because they are a wading bird and because they have a wide range of potential

prey items, including fish, insects, marine invertebrates, small mammals, reptiles and amphibians (Mccrimmon et al. 2011).

Our field collaborators captured AMOY and BLSK with decoy-noose and box traps and cannon-nets, BRPE with noose and padded leg-hold traps, and GREG with net guns (Mills and Ryder 1979; Crozier and Gawlik 2003; McGowan and Simons 2005, Herring et al. 2008). Clapper rails were captured by hand with night lighting from airboats as well as with drift fences leading to box traps. Seaside sparrows were captured with mist nets. Details of avian capture and sample collection protocols can be found in the reports cited above (http://www.doi.gov/deepwaterhorizon/index.cfm)

For AMOY, BLSK, BRPE and GREG, API sites included locations along coastal Louisiana, with five BRPE collected from coastal Mississippi, where exposure to oil from the Deepwater Horizon spill was likely (Figure 2). For these four species, reference sites included various locations along coastal South Carolina and Georgia, USA (Figure 3). Because CLRA and SESP species maintain small home ranges, reference sites for these two species included saline *Juncus* marshes, saline *Spartina* marshes, and brackish *Phragmites* marshes with no visible oil along coastal Louisiana, Mississippi, and Alabama (Figure 2). Conversely, API sites for CLRA and SESP included only marshes where visible oil was present along coastal Louisiana and Mississippi (Figure 2).

Visible oiling

Our field collaborators evaluated the majority of birds captured in both API and reference sites for evidence of visible oiling, and assigned an oiling score of none (0% of plumage affected

with visible oil), trace (<5% plumage affected), light (6-20% plumage affected) moderate (21-40% plumage affected) or heavy (> 40% plumage affected).

Ultraviolet-assisted oiling

Our field collaborators applied an ultraviolet (UV) light source (Labino compact PH135 UV spotlight, Labino AB Solna, Sweden) to the majority of birds captured in order to detect oiling that may have not been otherwise visible. Petroleum products are known to fluoresce under ultraviolet light (Colligan and LaManna 1993). Each bird was placed under an opaque covering to block out natural light, exposed to the UV light, and assigned an oiling score of none (0% of plumage affected), trace (<5% plumage affected), light (6-20% plumage affected) moderate (21-40% plumage affected) or heavy (> 40% plumage affected). The occurrence of UV-detectable oiling was assessed independent of visible oil assessments. As visible oil will fluoresce under UV illumination, birds grouped within the "UV oiled" designation included visibly oiled birds and a portion of non-visibly oiled birds.

Blood collection and sample handling

For AMOY, BLSK, BRPE, CLRA, and GREG our collaborators in the field collected blood from the medial metatarsal vein or superficial ulnar vein using 21 or 23G butterfly catheters deposited directly into lithium heparin and ethylenediaminetetraacetic acid (EDTA) vacutainers. Immediately following collection, field teams filled two heparinized hematocrit tubes for PCV analysis from the butterfly tubing. For SESP, blood was collected from the

superficial ulnar vein via venipuncture with a 27G needle and heparinized hematocrit tubes via capillary action.

Immediately after blood collection, we prepared new methylene blue-stained blood smears in the field to quantify Heinz bodies and reticulocytes as described below. Remaining blood samples were maintained on ice and transferred to the field laboratory within 12 hours of collection. Once in the field laboratory, our collaborators prepared two additional EDTA-treated blood smears for complete blood cell analysis using a standard two-slide technique (Aird 2010). Heparinized plasma was immediately separated from cells via centrifugation and transferred to cryovials. Heparinized whole blood and heparinized plasma were shipped under chain of custody overnight on ice to Avian and Exotic Clin Path Labs (Wilmington, OH) for complete blood cell count (CBC) and biochemical analyses. Plasma for ferritin and haptoglobin analyses was frozen and stored at -80 °C until processing. The packed cellular component of the blood was transferred to cryovials and stored at -80 °C until processing for PAH analyses.

Heinz bodies and reticulocytes

We prepared the new methylene blue stained slides within minutes of field collection for a subset of captured birds. To prepare these, we transferred and mixed 25ul of blood from the EDTA vacutainer to a 0.5ml centrifuge tube containing 25ul of new methylene blue stain. We allowed this combination to incubate for 20 minutes, and then prepared two blood smears via standard two-slide technique (Aird, 2010). We evaluated slides within 72 hours of collection. We identified Heinz bodies as dark-staining round bodies along the surface of erythrocytes, and identified reticulocytes as erythrocytes with reticular remnants encircling > 50% of the

circumference of the nucleus (Figure 4) (Johns et al. 2008). We counted 1000 erythrocytes under 1000X light microscopy, noting the number of cells affected by Heinz bodies as well as the number of reticulocytes. Individuals performing these analyses were blinded to oiling status, capture location, and results of other analyses.

PCV, Hb, MCHC, RBC, and MCV

Field teams processed samples for PCV (%) and Hb (g/dl) within 12 hours of collection. Packed cell volume was calculated using a standard hematocrit reader following centrifugation at 11,000 rpm for 5 minutes. Total Hb (g/dl) was quantified using a Hemocue Hb Analyzer Hb201, which relies on azidemethemoglobin reaction (Velguth et al. 2010). We calculated MCHC (g/dl) by dividing the total Hb by PCV (expressed as a proportion) (Latimer et al. 2003). Red blood cell count (RBC) was estimated via standard manual methodology using a hemocytometer (Campbell 1995). We calculated mean cell volume (MCV, in femptoliters or fL per cell) by dividing PCV (as a proportion) by RBC count (in cells/mm^3) and multiplying the quotient by 10^9. Individuals performing these analyses were blinded to oiling status, capture location, and results of other analyses.

Plasma haptoglobin and ferritin

We determined plasma haptoglobin levels using a standard microplate colorimetric assay following the manufacturer's specifications (Tri-Delta Phase RangeTM haptoglobin colorimetric bioassay, Biognosis Ltd., UK). This assay is based on the principle that free Hb exhibits peroxidase activity, which is inhibited at a low pH. Haptoglobin present in the plasma sample

combines with Hb and at a low pH preserves the peroxidase activity of the bound Hb, which is directly proportional to the amount of haptoglobin present in the specimen. We employed standard quality assurance procedures including analysis of standard reference materials, duplicate samples, method blanks, and laboratory control samples (red-shouldered hawk, RSHA, and bald eagle, BAEA, plasma) with each analytical batch.

We determined plasma ferritin using a standard colorimetric assay (Aoki et al. 1992, Troisi et al. 2007). This assay is based on the principle that thioglycolic acid can be used as a reducing agent for iron, and that bathophenanthroline disulfonic acid produces a reliable spectrophotometric change correlating to the concentration of ferritin in the plasma (Aoki et al. 1992). Ferritin standards derived from purified equine spleen ranged from 18.75 to 300 ng/ml. We prepared a protein precipitation solution (10% w/v trichloroacetic acid, 3% w/v HCl plus 40% v/v thioglycolic acid) and color reagent (1.5M sodium acetate containing bathophenanthroline disulfonic acid). We added twenty-five µl of protein precipitation solution to 25 µl of each prepared standard, samples, controls, and blanks. We then gently vortexed mixtures and incubated them at 56° C for 15 minutes, before cooling on ice. We then added 150 µl of color developing solution and allowed mixtures to incubate at 25° C for 5 minutes. We centrifuged these at 5000 rpm for 5 minutes, then transferred 150 µl of supernatant to microplate wells in duplicate and read the plate at 535 nm absorbance immediately. We employed standard quality assurance procedures including analysis of standard reference materials, duplicate samples, method blanks, and laboratory control samples (RSHA and BAEA plasma) with each

analytical batch. Individuals performing these analyses were blinded to oiling status, capture location, and results of other analyses.

Plasma biochemistry and white blood cell count

Avian and Exotic Clin Path Labs (Wilmington, OH) determined the following biochemistry parameters using standard spectrophotometric methodology: calcium (Ca), total protein, phosphorus (P), lactate dehydrogenase (LDH), creatine phosphokinase (CPK), chloride (Cl), uric acid (UA), glucose, cholesterol, and aspartate aminotransferase (AST). Standard quality assurance and quality control measures were employed. Individuals performing these analyses were blinded to oiling status, capture location, and results of other analyses.

White blood cell counts were determined via standard blood smear estimation methodology (Campbell and Ellis 2013). Wright's-stained slides were examined under 400X to estimate total white blood cell count, and individual leukocytes were classified as heterophils, lymphocytes, eosinophils, basophils and monocytes under 1000X magnification, (Thrall et al. 2004, Campbell and Ellis 2013). Thrombocytes were present on slides from all individuals, but this cell type was not enumerated because they were frequently clumped. At least 100 leukocytes were counted and only fields of view with even distributions of red blood cells were used. The proportion of each cell type was calculated based on the number of cells of that type divided by the total number of leukocytes.

Polycyclic aromatic hydrocarbons

The University of Connecticut's Center for Environmental Sciences and Engineering laboratory determined total PAH concentrations, as well as the concentration of naphthalene, acenaphthylene, acenaphthene, fluorine, anthracene, phenanthrene, fluoranthene, pyrene, chrysene, benzo-a-anthracene, benzo-b-fluoranthene, benzo-k-fluoranthene, benzo-a-pyrene, dibenzo-a-h-anthracene, benzoperylene, and indeno(1,2,3-cd) pyrene levels, all in ng/ml, using gas chromatography (Pleil et al. 2010). For each sample, approximately 0.1 to 0.5g of red blood cells were extracted in hexane, then vortex mixed before centrifugation. The extract was layered onto Sep-Pak Vac Alumina N 6cc (1g) cartridges (Waters, Inc. Milford, MA, USA) for sample purification. An internal standard (chrysene d-12) was added to bring the final volume to 0.50 mL with acetonitrile. Subsequently, samples were analyzed on the Acquity ultra performance liquid chromatography (UPLC, Waters, Inc. Milford, MA, USA) using a standard column (BEH C18 1.7µm, 2.1x50mm, Waters, Inc., Milford, MA, USA) equipped with photodiode, fluorescence, and tandem mass spectrometry (MS/MS) detectors. Peaks were identified and quantified using MassLynx software. Target analytes were compared to the retention times of the PAH standard mixture (Pleil et al. 2010).

Toxicological Endpoint Prioritization

The final data set resulted in 30 variables that could potentially be used to assess effects of oiling on birds. Although statistical comparison of all the variables could be undertaken, evaluation of significance is difficult due to problems associated with multiple comparisons (Westfall and Young, 1993, Beninger et al. 2012). When the number of tests is large and all tests are carried out with standard pre-set Type I error rates (alpha level), there is a high probability of

rejecting at least one test just by chance. To circumvent this problem, the number of variables was reduced by developing an *a priori* list of variables that were considered high priority for analysis. Following a thorough literature review, we created a list that categorized expected response to oil exposure as low, moderate, or high. These *a priori* decisions were made based on the likelihood of biological relevance to oil exposure extrapolated from existing literature on birds exposed to petroleum products. The list and associated references are provided in Table 2.

Statistical Analyses

We used SAS software (version 9.3 SAS Institute Inc., Cary, NC, USA), Microsoft Excel, or R 3.0.0 (Foundation for Statistical Computing, Vienna, Austria) for all analyses. Where appropriate, we evaluated normality and homogeneity of variance using Shapiro-Wilk and Levene's tests, respectively.

We relied on univariate analyses throughout this report for three reasons. First, the data were not normally distributed, which precluded the use of normal multivariate analyses (Oja 1981). Second, classical multivariate statistical methods for nonparametric data such as principal components analysis (PCA), multivariate multiple regression, canonical correlation analysis, and factor analysis are based on the sample mean vector and covariance matrix (Johnson and Wichem 2002). The data set has a number of outlying observations and we found residuals were not normally distributed. Hence, we expected standard multivariate methods to excessively weight extremes resulting in potentially misleading inferences. The third and most important reason for not using multivariate analyses is that the majority of individual birds from this dataset have missing values for one or more variables (Little and Rubin 1987). Accounting for

these missing data in a multivariate approach would require either removing all birds with missing data, which would greatly reduce the sample size, or to use imputation. Imputation methods may be problematic for several reasons including: additional assumptions are made that can lead to bias when violated; few imputation models can effectively deal with datasets containing both continuous and categorical variables; and multiple imputation models are required for independent and dependent variable imputation (Rubin 1996, 2003). For all of these reasons, we ultimately relied on univariate models to conduct independent analyses of the suite of physiological endpoints with each of the following variables: visible oiling, species, UV oiling, and total PAH concentrations To account for the lack of independence of physiological responses compared in our univariate models, we applied a conservative α to assess significance. We set a significance level of $\alpha \leq 0.01$ for testing all of the high priority variables identified in the *a priori* prioritization. In cases where $\alpha > 0.01$ and $\alpha \leq 0.05$, we made note of statistically marginal significance for high priority variables. We set a more conservative significance level of $\alpha \leq 0.001$ for all low and moderate priority variables (Table 2). With the large number of physiological variables included in this study, this conservative approach reduces the risk of identifying significant effects due to chance alone.

Circulating PAH concentrations are likely species-dependent because of differences in behavior that influence risk of exposure to oil as well as physiological differences in absorption and metabolism of petroleum. Likewise, the physiological responses to PAHs, such as changes in erythrocytes or biochemical variables, are also likely species-dependent. Therefore, we first evaluated whether species differed in circulating PAH levels, visible oiling, UV oiling, and the suite of physiological variables for each capture site type (reference and API locations) using

Kruskal-Wallis tests with species as the independent variable. Because these analyses revealed significant differences in several parameters among species (see results), we next analyzed each species separately to more closely examine the effects of oiling on physiological parameters.

To determine the effects of visible oiling on Heinz body formation, reticulocytes, PCV, Hb, MCHC, RBC, MCV, ferritin, haptoglobin, and the clinical biochemistry variables Ca, total protein, P, LDH, CPK, Cl, UA, cholesterol, and AST we used Kruskal-Wallis separately for each species with subsequent post-hoc analysis to compare capture site type/oiling classifications (using the SAS Multtest procedure). Because of a limited range of severity of visible oiling (Table 3), we classified birds for statistical models as either reference, API with visible oil, or API with no visible oil.

To determine the effects of UV-detectable oiling on the same suite of variables we used Kruskal-Wallis separately for each species with subsequent post-hoc analysis to compare capture site type/oiling classifications (using the SAS Multtest procedure). Because there was a wider range of UV oiling severity than visible oiling severity, we classified individual birds for statistical models as either reference, or API with no, trace, light, moderate, or heavy UV oiling. This approach allowed us to examine whether the enhanced resolution provided by UV oil detection (compared to visible oil detection) allowed for more clear discrimination of possible dose-response relationships between degree of oiling and adverse effects.

To determine the effect of circulating PAHs on the same suite of variables we used two different approaches. Due to the large number of analytical non-detects, we first grouped individuals as PAH present or PAH absent (i.e., below detection limit of 5 ng/g) and used Wilcoxon rank-sum (Mann-Whitney) tests for each species to determine whether PAH presence

influenced physiologic variables. Second, we used Spearman correlations to determine the strength of a species-specific linear relationship between total PAH concentration and all of the physiologic variables measured.

Statistical evaluations of physiological measures as a function of visible and UV-detectable oiling were reported in a separate table for each priority response. Both significant ($p \leq 0.01$) and marginally significant ($0.05 \geq p > 0.01$) interactions were reported. For purposes of data visualization, significant and marginally significant interactions are illustrated under a single level of significance, $p \leq 0.05$, in the figures.

We excluded CLRA from many of the statistical analyses as a function of visible and UV oiling due to a paucity of birds. These analyses included Heinz body counts, reticulocyte counts, PCV, Hb, MCHC, MCV, haptoglobin, ferritin and circulating PAH concentration. Also, our field collaborators collected no UV oiling data in AMOY from reference areas; therefore, this species was excluded from statistical analyses based on UV oiling status.

There was a modest time delay between blood collection in the field and centrifugation at the field laboratory, which could result in spuriously lowered blood glucose concentrations. Therefore, this endpoint was not included in any analyses (Latimer et al. 2008). This delay in centrifugation is not likely to significantly impact other biochemical endpoints (Latimer et al. 2008)

RESULTS

Species differences

In reference areas, Kruskal-Wallis tests revealed differences among species in reticulocytes, ferritin, PCV, Hb, MCV, WBC, Ca, Phos, LDH, CPK, Cl, UA, Chol, and AST but

not in Heinz bodies, haptoglobin, MCHC, or total PAH (Table 4). In API sites, we found significant differences among species in total PAH, Heinz bodies, reticulocytes, haptoglobin, ferritin, PCV, Hb, total solids, MCV, WBC, Ca, Phos, LDH, CPK, UA, Chol, and AST but not in MCHC or Cl (Table 4). In our targeted API areas, visible oiling rates were highest in AMOY and GREG, while UV oiling rates were highest for CLRA and GREG (Figure 5) and, except for AMOY, proportions of UV oiled birds were higher than visibly oiled birds (see UV Fluorescence section in Results, below, for description of AMOY findings). These results indicate that there are species differences present in the majority of variables within both reference and API sites, and that within species comparisons are necessary to distinguish effects of oiling on avian physiology.

Visible oiling

We found no light, moderate, or heavily oiled birds in the reference areas; however, we found 7 GREGs from reference areas with trace quantities of visible oiling. To be true to our *a priori* categorization of birds, we retained these birds in the reference group for our statistical analyses. By retaining these trace-oiled birds in the reference group, we were conservative in our analysis, because in our best professional judgment we expected that this would cause reference values to be closer to those of birds in the API category. In APIs, we found numerous birds with trace and light oiling, but we captured no moderately or heavily visibly-oiled birds (Table 3). In birds captured from API sites visible oiling rates ranged from 5% in SESP to 75% in AMOY (Figure 5).

Heinz bodies and reticulocytes

Birds from API sites, both those with oil and those without, had higher Heinz body counts than birds from reference sites (Table 5, Figure 6). Kruskal-Wallis analysis among the site categories (birds from reference sites, API without visible oil, and API with visible oil) revealed significant differences in Heinz body occurrence in BLSK, BRPE and GREG (Table 6). Post hoc analyses demonstrated that API oiled BRPE had marginally significant higher Heinz body counts than reference birds, but neither differed from API non-visibly oiled birds (Table 6). Similarly, API oiled GREG had significantly higher Heinz body counts than birds from reference sites but neither differed from API non-visibly oiled birds (Table 6). Oiled BLSK from API sites also had significantly higher Heinz body counts than reference birds, and in addition they had significantly higher Heinz body counts than API non-visibly oiled birds.

Birds from the API had higher reticulocyte counts than birds from reference sites (Table 7, Figure 7). Kruskal-Wallis analysis among sites (reference, API without visible oil, and API with visible oil) revealed significantly different reticulocyte counts in AMOY, BLSK, BRPE and GREG. Post hoc analyses revealed that API oiled and API non-visibly oiled BLSK and BPRE had significantly higher reticulocyte counts than reference birds (Table 8). Oiled AMOY and GREG had significantly higher reticulocyte counts than reference birds while neither differed from API non-visibly oiled birds (Table 8).

Packed cell volume, Hemoglobin, and Mean corpuscular hemoglobin concentration

Birds from API sites had lower PCV values than birds from reference sites (Table 9, Figure 8). We found a significant difference among sites (reference, API without visible oil, and

API with visible oil) in PCV in AMOY, BRPE, and GREG (Table 10). Post hoc analyses revealed that API oiled and API not visibly oiled AMOY, BRPE, and GREG had significantly lower PCV values than reference birds (Table 10).

Birds from API sites also had lower Hb concentration than birds from reference sites (Table 11, Figure 9). We found significant differences among sites (reference, API without visible oil, and API with visible oil) in Hb concentrations in BRPE and GREG (Table 12). Post hoc analyses revealed that API oiled and API not visibly oiled BRPE had significantly lower Hb concentration than reference birds (Table 12). Similarly, API oiled and API not visibly oiled GREG had significantly lower Hb concentrations than birds from reference sites (Table 12).

When PCV and Hb were considered together to calculate mean corpuscular hemoglobin concentration (MCHC), birds from API had similar MCHC to birds from reference sites (Table 13 and 14, Figure 10).

Red blood cell count and mean cell volume

Birds from API had lower RBC counts than birds from reference sites (Table 15, Figure 11). We found a significant difference among sites (reference, API without visible oil, and API with visible oil) in RBC counts in GREG (Table 16). Post hoc analyses revealed that GREG from reference areas had significantly higher RBC counts than birds from API areas with no visible oiling, and marginally higher RBC counts than birds from API sites with visible oil (Table 16).

Birds from API had varied MCV compared to birds from reference sites (Table 17, Figure 12). We found a significant difference among sites (reference, API without visible oil,

and API with visible oil) in MCV in BRPE and GREG (Table 18). Post hoc analyses revealed that BRPE from API sites with and without visible oiling had lower MCV than reference birds, while GREG from API sites with visible oil had lower MCV and without visible oil had marginally lower MCV than reference birds (Table 18).

Haptoglobin and Ferritin

We found no significant relationship between visible oiling and haptoglobin concentration in AMOY, BRPE, or GREG (Table 19, Figure 13). However, in BLSK we found a marginally significant difference among sites (reference, API without visible oil, and API with visible oil) in haptoglobin concentration. Black skimmers from API sites with no visible oil had lower haptoglobin concentrations than reference sites (Table 20).

We found no significant relationship between visible oiling and ferritin concentration in AMOY, BLSK or BRPE (Table 21, Figure 14). We found a significant difference among sites (reference, API without visible oil, and API with visible oil) in ferritin concentration in response to visible oiling in GREG (Table 22). Post hoc analyses revealed that GREG from reference areas had significantly higher ferritin than birds from API areas with visible oiling and API areas with no visible oiling (Table 22).

Polycyclic aromatic hydrocarbons

In AMOY, BLSK, and BRPE, birds from API sites tended to have higher circulating concentrations of PAH than birds from reference sites, however, the results were not significantly different (Figure 15).

Biochemical profile

We found no significant relationships between visible oiling and Ca, total protein, P, LDH, CPK, UA, and AST. However, we found a significant difference among sites (reference, API without visible oil, and API with visible oil) in cholesterol in BRPE and GREG (Table 23). Post hoc analysis revealed that birds of both species from API sites with and without visible oil had lower cholesterol than reference sites ($p < 0.001$). We also found significant difference among sites in chloride in GREG with visible oiling (Table 23). Post hoc analyses revealed GREG with and without visible oiling in API sites had higher chloride than reference sites ($p < 0.001$)

UV fluorescence

Based on UV inspection, we found no moderate or heavily oiled birds in the reference areas; however, we found 27 GREGs from reference areas that had trace quantities and one GREG from a reference area that had light quantities of UV oiling. These birds were retained in the reference group for these analyses for the same reasons outlined, above, under "Visible Oiling". In APIs, a greater proportion of study birds was diagnosed as oiled using UV fluorescence than visible inspection (Figure 5). The exception was AMOY, with a lower UV oiling rate than visibly inspected birds. This difference reflects a small sample size for the UV group compared to the visible oil birds (13 vs 43, Tables 3 and 24). While 46% of the 13 UV-inspected AMOY had evidence of UV oiling, visual inspection of these individuals demonstrated that only 31% were visibly oiled, consistent with all other species where a greater proportion of

oiled birds was detected with UV than visual detection. Of the UV oiled birds, we found many birds with trace and light UV oiling, 8 birds with moderate UV oiling, and 2 birds with heavy UV oiling (Table 24). Birds that were assessed for UV oiling were also assessed for visible oiling, however, not all birds that had visible assessment were assessed for UV fluorescence.

Heinz bodies and reticulocytes

Birds with UV oiling had higher Heinz body counts than birds from reference sites (Table 25, Figure 16). We found a significant difference among degree of UV oiling from API and reference sites in Heinz body formation in BLSK. Post hoc analyses revealed that BLSK with trace and light oiling had significantly higher Heinz body counts than BLSK from reference areas. Further, lightly UV oiled BLSK had higher Heinz body counts than API birds with no UV oiling and API birds with trace UV oiling (Table 26). BRPE with trace UV oiling had significantly higher Heinz body counts than reference birds (Table 26). Similarly, GREG with light UV oiling had higher Heinz body counts than GREG from reference areas and GREG with trace UV oiling. Further, GREG with light UV oiling had significantly higher Heinz body counts than API birds with no UV oil (Table 26).

We found that reticulocyte counts increased with severity of UV oiling and were higher in birds from API sites than birds from reference (Table 27, Figure 17). We found a significant difference among degree of UV oiling from API and reference sites in reticulocyte formation in BLSK, BRPE, and GREG (Table 27). Post hoc analyses revealed that BLSK with trace and light oiling had significantly higher reticulocyte counts than BLSK from reference areas. Brown pelicans with trace UV oiling had significantly higher reticulocyte counts than reference birds

(Table 28). Similarly, GREG with trace and light UV oiling had higher reticulocyte counts than GREG from reference areas (Table 28).

Packed cell volume, hemoglobin and mean corpuscular hemoglobin concentration

We found PCV were lower in birds from API sites than birds in reference sites (Table 29). We found a significant difference in PCV in BRPE and GREG with UV oiling (Table 30, Figure 18). Post hoc analyses revealed that BRPE from API sites with no UV oiling and from API sites with trace UV oiling had significantly lower PCV than reference birds (Figure 18, Table 30). Similarly, GREG from API sites with no, trace, and light UV oiling had lower PCV than GREG from reference areas (Table 30).

Hemoglobin was lower in birds from API sites than those from reference sites. We found a significant difference in Hb in BREP, and GREG with UV oiling (Table 31, Figure 19). Post hoc analyses revealed that BRPE from API sites with no UV oiling and from API sites with trace UV oiling had significantly lower Hb than reference birds (Figure 19, Table 32). Similarly, GREG from API sites with trace UV oiling had lower Hb than GREG from reference areas (Table 32). We found no statistical significance in MCHC between birds with and without UV oiling (Table 33).

Red blood cell count and mean cell volume

We found a marginally significant difference in RBC in GREG with UV oiling (Table 34, 35, Figure 20). Post hoc analyses revealed that GREG from API sites with no and light UV oiling had lower RBC than reference birds (Table 35 Figure 20*)*. We found marginally lower

MCV in BLSK with trace UV oiling than birds from reference areas and API with no visible oil (Table 36). We found lower MCV in BRPE and GREG with trace UV oil than those from reference areas (Table 37.)

Haptoglobin, Ferritin, and Polycyclic aromatic hydrocarbons

We found no statistical difference in haptoglobin concentration in birds with varying degrees of UV oiling (Table 38). However, we found a significant difference in ferritin in GREG with UV oiling (Table 39, 40, Figure 21). Post hoc analyses revealed that GREG from API sites with no UV oiling and those with light UV oiling had significantly lower ferritin than reference birds (Table 40, Figure 21). We found no statistical difference in total PAH concentrations between birds with varying degrees of UV oiling.

Biochemical profile

We found no significant relationships between UV oiling and Ca, total protein, P, LDH, CPK, UA, and AST. However, we found a significant difference in cholesterol in BRPE and GREG with UV oiling (Table 41). Post hoc analysis revealed that BRPE from API sites with trace UV oiling and API without UV oiling had lower cholesterol ($p < 0.001$) than BRPE from reference areas. Similarly, GREG with trace and light UV oiling had lower cholesterol than GREG from reference sites ($p<0.001$ in both cases). Chloride was also significantly affected by UV oiling in GREG (Table 41). Post hoc analyses revealed that GREG from API sites with trace UV oiling had higher chloride ($p < 0.001$) than reference birds.

Circulating polycyclic aromatic hydrocarbon concentrations

We found that PAHs were present in 65 birds and undetectable in 302 birds (Table 42). The concentration of total PAHs in the blood ranged from 0 to 253.6 ng/ml. Wilcoxon analysis revealed no significant relationship between PAH concentration and Heinz bodies, reticulocytes, PCV, Hb, MCHC, RBC, or ferritin. We found that BLSK with PAHs present in the blood had lower haptoglobin than those with no PAHs ($z = -1.79$, $p = 0.037$). Black skimmers with PAHs in their blood had higher MCV than those with no PAHs ($z = 2.13$, $p = 0.016$). American oystercatchers ($z = 1.79$, $p = 0.037$), CLRA ($z = 1.68$, $p = 0.047$), and GREG ($z = 2.08$, $p = 0.018$) with PAHs in their blood had marginally higher WBC counts than those with no PAH (Figure 22).

Spearman correlation revealed no significant relationships for Heinz bodies, reticulocytes, PCV, Hb, MCHC, RBC, MCV, haptoglobin or ferritin and PAH concentrations (in all cases $p > 0.01$).

DISCUSSION

The Deepwater Horizon spill released a large volume of crude oil into the Gulf of Mexico (McNutt et al. 2012). While there were thousands of dead birds found in the weeks following the disaster, there were many more that were likely exposed to oil but did not immediately succumb (Peterson et al. 2003, USFWS 2011). Here, we demonstrate that birds with small amounts of oil present on their feathers can suffer from oxidative injury to erythrocytes, decreased numbers of circulating erythrocytes, and show evidence of a regenerative hematological response in the form of increased reticulocytes. Together these findings provide considerable associative evidence between oil exposure and hemolytic anemia (Table 34).

The presence of Heinz bodies in AMOY, BLSK, BRPE, and GREG with visible oil from impacted sites indicated that oxidative injury to erythrocytes had occurred in each of these species. Further, this oxidative injury was either the result of recent onset or was ongoing, as cells with Heinz bodies undergo lysis and/or are rapidly removed from circulation (Campbell and Ellis 2007). Conversely, in all of the individual AMOY, BLSK, BRPE, CLRA and GREG from the reference sites that we assessed for Heinz bodies (n = 176) we found only a single GREG with Heinz bodies present, indicating that oxidative injury as evidenced by Heinz bodies is quite uncommon in reference populations of these species. While we found no Heinz bodies in CLRA from either API or reference sites, our sample size of visibly oiled birds from this species was small.

The presence of Heinz bodies combined with increased reticulocytes found in oiled AMOY, BLSK, BRPE, and GREG from API sites suggests a regenerative response to oxidative insult and hemolysis. Additionally, AMOY, BRPE and GREG from impacted sites had

decreased PCV. These three features—presence of Heinz bodies, decreased PCV and increased reticulocytes—are indicative of oxidative injury, anemia, and a physiological regenerative response. This physiologic cascade can cause a decrease in oxygen availability to tissues (Latimer et al. 2003). This can induce muscle fatigue, lethargy, decreased energy availability for metabolic processes, adverse reproductive impacts, and may have implications for survival and fitness (Butler et al. 1986, Piersma et al. 1996, Walton et al. 1997, Ots et al. 1998, Hylton et al. 2006).

We found hematological injury not only in birds from API sites with evidence of oiling, but also in birds from API sites without evidence oiling when compared to reference birds. This finding is important, as it suggests that birds present in the API were experiencing oxidative injury even without evidence of visible oil exposure or UV fluorescence. While oil that was previously present on the feathers may have been preened and ingested, oil exposure can also occur from foraging and contaminated prey (Shields 2002).

Our results indicate that there are species differences in the majority of physiological variables within both reference and API sites (Table 4), highlighting the importance of within-species comparisons to distinguish effects of oiling on avian physiology. These species differences are not surprising, as variations across species have been noted for both hematological and plasma biochemistry values in healthy birds (Gee et al. 1981, Harr 2002). In addition to inherent physiological variations among species, differences in foraging strategies, habitat preferences, and behaviors may play a role in differences in both exposure and effects among species. For example, in this study we found that GREG were the only species with reference individuals that had evidence of visible oiling (n=7, Table 3). Similarly, other than one

CLRA with trace fluorescence, GREG were the only species with reference individuals with evidence of UV fluorescence (n=28, Table 23). Among species in this study, GREG are more apt to frequently forage in man-made drainage ponds and pooled, standing water from residential or industrial run-off which may contain petroleum waste (McCrimmon et al. 2011). Therefore, reference populations of this species may be at higher risk for exposure to oil than other species examined in this investigation. Despite species differences in foraging, roosting strategies and other behaviors, the physiological sequela to oxidative damage to erythrocytes is likely consistent across species (Harvey and Kaneko 1977).

The use of hand-held ultraviolet lights can enhance the sensitivity of visible oiling assessments, as even trace amounts of oil have been shown to fluoresce (Chase et al. 2005). Here, UV assessment identified oil on many birds that appeared to be oil-free on visual exam; 266 birds with no detectable oil noted on visual assessment had trace UV fluorescence present. Further, UV fluorescence provided a wider range of oiling severity than visible oiling because of enhanced resolution between oiling categories. The data presented here provide some evidence, though not always statistically significant, of increased physiological effects with heavier UV oiling in some species (Figs. 16, 17, 18, 19). However, ultraviolet assessment revealed similar relationships with the suite of physiological response variables as visible oiling assessment, which is likely due in part because the vast majority of birds with visible oiling also had evidence of UV fluorescence. In this study, 78% of birds that were assessed for visible oiling or UV fluorescence were evaluated for both. Taken together, our results suggest that UV assessments can be useful in identifying birds with very small amounts of oil, but that this UV

methodology may not improve the ability to detect relationships with physiological injury over the use of visual oil assessment alone.

Haptoglobin and ferritin have been suggested as markers for oxidative injury from crude oil exposure (Troisi et al. 2007). However, there have been mixed results concerning the relationship between haptoglobin and oil exposure (e.g., Prichard et al. 1997, Troisi et el. 2007). We found a marginal significant relationship between haptoglobin in reference and API BLSK (Table 20). Additionally, we found a trend towards decreased haptoglobin in oiled individuals of all species when compared to reference values (Executive Summary Table 1). Haptoglobin levels have been shown to vary widely in both reference and exposed individuals, and species differences in normal levels occur commonly (Prichard et al. 1997, Ben-David et al. 2001). Additionally, circulating haptoglobin is known to increase in response to inflammation (Delers et al. 1988). Therefore, it is possible that oil-induced inflammation is increasing circulating haptoglobin while hemolysis is consuming it, leading to a limited net effect. We found that ferritin was related to oil exposure only in GREG and not in the other species evaluated in this study. Ferritin functions to store iron, but it also functions to quench free radicals, such as hydrogen peroxide, and serves as an acute phase protein (Beck et al. 2002). Moreover, ferritin has been found to vary by age and species (Theil 1987). Most importantly, the majority of ferritin within the body is found within cells, not in plasma where it has been historically measured in wild birds (Prichard et al. 1997, Seiser et al. 2000 Ong et al. 2005, Troisi et al. 2007). Our results here suggest that these plasma proteins may not be useful specific biomarkers of trace and light oil exposure in contrast with previous findings in heavily oiled birds (Troisi et al. 2007).

While RBC counts were significantly lower in API sites, the difference between RBC in API and reference sites was not as dramatic as PCV results. Additionally, MCV did not differ as predicted *a priori.* These results may have occurred because while PCV was determined in a field laboratory shortly after collection, RBC was determined at a commercial laboratory after shipping. The time delay from collection to RBC determination may have resulted in evaporation of plasma leading to an artifactually higher RBC count (Latimer et al. 2003). Consequently, because MCV is the result of PCV/RBC, an artifactually elevated RBC would cause a relative decrease in MCV. Mean corpuscular hemoglobin concentration is an important parameter for distinguishing non-regenerative anemia (e.g., iron deficiency, chronic disease), which have below normal MCHC, from regenerative anemia (e.g., hemolysis), which have stable or artifactually elevated MCHC (Latimer et al. 2003). In all species, MCHC was higher in birds from API sites than birds from reference sites, though the results were not statistically significant. These results are consistent with the *a priori* prediction.

We found limited significant associations between oil and other plasma biochemical parameters, which was consistent with our *a priori* hypotheses. The lower cholesterol that we found in BRPE and GREG from API sites compared to reference sites is consistent with findings in yellow-legged gulls exposed to the Prestige oil spill (Alonso-Alvarez et al. 2007). Cholesterol has been shown to be a potential index of individual condition in some species (Alonso-Alvarez et al. 2007, Alonso-Alvarez and Velando 2003). Therefore, it is possible that a combination of factors resulting from oil exposure resulted in decreased cholesterol, body condition, or both in BRPE and GREG. The elevated chloride found in GREG from API sites may be consistent with previous findings of electrolyte disturbances, particularly osmoregulatory impairment that has

been reported in other species including mallards, guillemots, and herring gulls exposed to crude oil (Miller et al. 1978, Peakall et al. 1980, Eastin and Rattner 1982).

In contrast to visible and UV oiling, we found no correlation between circulating total PAH concentrations and physiological variables. Likewise, we found no correlation between visible or UV oiling and total PAH concentrations. This lack of association may be due to a combination of factors. For example, we measured PAHs only in the cell fraction of the blood, which may not capture the total burden present in circulation. Additionally, PAHs are rapidly metabolized *in vivo*. This rapid break down of parent compounds is the most frequently documented reason for the lack of correlation between PAH and physiological endpoints (Short and Springman 2007). Finally, the oxidative injury to erythrocytes and subsequent hemolytic anemia is triggered by metabolites of PAHs; therefore, measuring the parent PAHs themselves, which are rapidly metabolized, may not adequately identify specific metabolic toxicants (Lo and Sandi et al. 1978).

While circulating PAH concentrations may be important for identifying recent exposure and understanding dose-response relationships, the methodology for quantifying blood PAH levels is time consuming, expensive, and not always practical in field studies, particularly if large sample sizes are desirable. Subjective visible oiling, on the other hand, can be determined quickly and inexpensively following only minimal personnel training. We demonstrated here that trace and light visible oiling are correlated with adverse physiological effects, such as oxidative hemolysis and decreased PCV.

In summary, our results demonstrate that birds exposed to oil from the *Deepwater Horizon* spill had evidence of oxidative injury to erythrocytes, decreased numbers of

erythrocytes in circulation, and evidence of an erythrocytic regenerative response. These changes are consistent with oxidative hemolytic anemia caused by exposure to oil. Although we did not clearly establish a dose-response relationship here, the physiological implications of Heinz body formation and their relationship to petroleum exposure make them an important parameter for health assessments following oil spill events, as Heinz body formation in birds is a pathological abnormality. In wild bird populations anemia has been shown to cause decreased survival (e.g., Yorinks and Atkison 2000) and has been linked to increased stress and immunosuppression (Applegate and Beaudion 1970) as well as decreased reproductive success (Hatch et al. 2010). These repercussions of anemia have obvious implications for individual fitness, and suggest that sublethal physiological injury associated with modest oil exposure may have important negative long-term repercussions for individuals.

Methodologically, our results also suggest that visible oiling is as good or better predictor of physiological impact than circulating PAH levels, and we suggest that visible oil evaluation should be a key component in future oil-spill events. Additionally, our work demonstrates that proper new methylene blue staining to enumerate Heinz bodies and reticulocytes is an important technique that can be applied under field conditions during petroleum-associated wildlife incident investigations (Fallon et al. 2013).

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Executive Summary Table 1

Kruskal-Wallis results summarized for high priority variables including Heinz bodies, reticulocytes, packed cell volume (PCV), hemoglobin (Hb), mean corpuscular hemoglobin concentration (MCHC), mean cell volume (MCV), haptoglobin, and ferritin found in American oystercatcher (AMOY), black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG). Percent differences calculated from the mean values from birds from reference areas and birds from API sites with visible oil relative to the reference mean. Directionality of the means is compared against *a priori* prediction. Asterisk represents significance ($p \leq 0.01$) and ** indicates marginal significance ($0.05 \geq p > 0.01$). N/A indicates that results were not determined for this variable in a particular species due to small sample size. Percent difference for Heinz bodies could not be calculated because no Heinz bodies were found for any reference birds except one GREG. Details of *a priori* predictions are in Table 2.

		AMOY			BLSK			BRPE			GREG		
Variable	*A priori* prediction	p value	Percent difference	Consistent with *a priori* prediction?	p value	Percent difference	Consistent with *a priori* prediction?	p value	Percent difference	Consistent with *a priori* prediction?	p value	Percent difference	Consistent with *a priori* prediction?
Heinz bodies	Increase	0.267	N/A	**Yes**	<0.001*	N/A	**Yes**	0.024**	N/A	**Yes**	0.003*	9644%	**Yes**
reticulocytes	Increase	<0.001*	40.16%	**Yes**	0.004*	25.63%	**Yes**	<0.001*	47.38%	**Yes**	0.010*	27.11%	**Yes**
PCV	Decrease	<0.001*	-11.54%	**Yes**	0.164	-3.67%	**Yes**	<0.001*	-13.83%	**Yes**	<0.001*	-19.02%	**Yes**
Hb	Decrease	N/A	N/A	N/A	0.876	-1.08%	**Yes**	0.002*	-10.29%	**Yes**	<0.001*	-12.61%	**Yes**
MCHC	Increase	N/A	N/A	N/A	0.878	3.31%	**Yes**	0.134	3.42%	**Yes**	0.195	7.33%	**Yes**
RBC	Decrease	N/A	N/A	N/A	0.613	-2.59%	**Yes**	0.561	-1.55%	**Yes**	0.003*	-7.77%	**Yes**
MCV	Increase	N/A	N/A	N/A	0.360	-5.97%	No	0.002*	-15.39%	No	0.004*	-9.38%	No
Haptoglobin	Decrease	0.319	-7.03%	**Yes**	0.014**	-24.97%	**Yes**	0.762	-14.73%	**Yes**	0.618	-4.34%	**yes**
Ferritin	Increase	0.073	-32.58%	No	0.568	-6.87%	No	0.184	-41.85%	No	<0.001*	-44.29%	No

Table 1
Number of American oystercatcher (AMOY), black skimmer (BLSK), brown pelican (BRPE), clapper rail (CLRA), great egret (GREG) and seaside sparrow (SESP) captured from reference areas and areas of potential impact.

	Reference	API	Total
AMOY	43	43	86
BLSK	89	123	212
BRPE	51	101	152
CLRA	88	108	196
GREG	48	57	105
SESP	104	401	505
All	423	833	1256

TABLE 2
A priori prioritization of hematological and biochemical values in birds potentially exposed to petroleum products.

VARIABLE	Priority	Predicted response to polycyclic aromatic hydrocarbon exposure	Biological Relevance	References
Heinz bodies	High	Increased number of circulating erythrocytes containing Heinz body inclusions	Formation may result from polycyclic aromatic hydrocarbon-induced oxidative injury to hemoglobin in erythrocytes; pathognomonic for oxidative injury to erythrocytes	Leighton et al. 1983; Fry and Lowenstine 1985; Leighton 1986; Couillard and Leighton 1993; Yamato et al. 1996; Troisi et al. 2007
reticulocytes	High	Increased number in circulation	Increase in the number of these immature erythrocytes occurs with regenerative hemolytic anemia.	Leighton et al. 1983; Fry and Lowenstine 1985; Leighton 1986; Couillard and Leighton 1993; Yamato et al. 1996; Troisi et al. 2007
packed cell volume (PCV)	High	Decreased percentage	This percentage of erythrocytes in blood is decreased in anemia. This value can decrease from hemolysis, blood loss, or decreased red blood cell production.	Leighton et al. 1983; Fry and Lowenstine 1985; Leighton, 1986; Couillard and Leighton 1993; Yamato et al. 1996; Troisi et al. 2007
hemoglobin (Hb)	High	Decreased concentration	Transport molecule for oxygen that is released from erythrocytes during hemolysis. This decreases during hemolysis and in anemia caused by blood loss.	Latimer et al. 2003

TABLE 2
A priori prioritization of hematological and biochemical values in birds potentially exposed to petroleum products.

VARIABLE	Priority	Predicted response to polycyclic aromatic hydrocarbon exposure	Biological Relevance	References
mean corpuscular hemoglobin concentration (MCHC)	High	Stable to increased	This calculated value of the quantity of Hb per cell (=Hb/PCV) is stable or artifactually increased during hemolysis, but is decreased during anemias of decreased erythrocyte production.	Latimer et al. 2003
red blood cell count (RBC)	High	Decreased number	Number of circulating erythrocytes estimated using hemocytometer. Can decrease from hemolysis, blood loss, or decreased red blood cell production. Positively correlated with PCV.	Yamamoto et al. 1996
mean cell volume (MCV)	High	Increased mean volume	Calculated as PCV/RBC, young erythrocytes tend to be larger in diameter, thus birds with a regenerative response to hemolytic anemia should have higher MCV.	Latimer et al. 2003
haptoglobin	High	Decreased concentration	Functions to sequester free hemoglobin resulting from hemolysis. May decrease from hemolysis due to consumption of available stores.	Troisi et al. 2007
ferritin	High	Increased concentration	Iron storage protein that increases in circulation in response to inflammation and hemolysis.	Troisi et al. 2007

TABLE 2
A priori prioritization of hematological and biochemical values in birds potentially exposed to petroleum products.

VARIABLE	Priority	Predicted response to polycyclic aromatic hydrocarbon exposure	Biological Relevance	References
total solids	Moderate	Stable to increased	Hemolysis results in loss of erythrocytes but not plasma solids. Dehydration and inflammation can cause an increase.	Latimer et al. 2003; Alonso-Alvarez et al. 2007
white blood cell count	Moderate	Increased	Total white blood cell count increases during inflammatory response. Hemolysis results in inflammation and glucocorticoid release.	Latimer et al. 2003; Campbell and Ellis 2007
cholesterol	Moderate	Stable or decreased	Plasma cholesterol level has been reported as an index of individual condition in certain species. In some species, has been found to be lower in birds exposed to crude oil.	Alonso-Alvarez et al. 2002b
aspartate aminotransferase (AST)	Moderate	Increased	Hepatocellular injury enzyme, but can also be elevated in skeletal muscle injury; increased levels with normal CPK indicate liver insult. Elevations have been demonstrated in birds exposed to PAH.	Seiser et al. 2000; Golet et al., 2002; Harr 2002.
creatine phosphokinase (CPK)	Moderate	Stable	Increases in response to muscle trauma and is required to interpret whether changes in AST are related to muscle or liver insult.	Lumeij and Westerhof 1987

TABLE 2
A priori prioritization of hematological and biochemical values in birds potentially exposed to petroleum products.

VARIABLE	Priority	Predicted response to polycyclic aromatic hydrocarbon exposure	Biological Relevance	References
heterophils	Low	Increased	Inflammatory and stress responses result in increased heterophil numbers and percentage.	Weiss and Wardrop 2011
lymphocytes	Low	Stable to decrease	Stress responses results in decrease in lymphocyte numbers and percentage.	Weiss and Wardrop 2011
eosinophils	Low	Stable or increased	May increase during inflammatory response; increases often associated with immune-mediated or parasitic diseases.	Weiss and Wardrop 2011
monocytes	Low	Stable or increased	May increase during chronic inflammatory and stress response.	Weiss and Wardrop 2011
basophils	Low	Stable or increased	The role of avian basophils is poorly understood. They may increase during inflammatory responses, associated with immune-mediated or parasitic diseases.	Weiss and Wardrop 2011
calcium	Low	Stable to increased	Proposed to be increased with hemoconcentration from dehydration.	Alonso-Alvarez et al. 2007; Hochleithner M. 1994.

TABLE 2
A priori prioritization of hematological and biochemical values in birds potentially exposed to petroleum products.

VARIABLE	Priority	Predicted response to polycyclic aromatic hydrocarbon exposure	Biological Relevance	References
total protein	Low	Stable to Increased	Hemolysis results in loss of erythrocytes but not plasma solids. Dehydration and inflammation can cause an increase.	Latimer et al. 2003
phosphorus	Low	Stable to decreased	Proposed to be reduced in birds exposed to polycyclic aromatic hydrocarbon-induced injury, but mechanism is unclear.	Alonso-Alvarez et al. 2007
lactate dehydrogenase	Low	Increased	Non-specific enzyme that increases with hepatic insult or injury to other tissues	Latimer et al. 2003
chloride	Low	Stable to increased	Electrolyte that can increase in states of dehydration.	Latimer et al. 2003
uric acid	Low	Stable or increased	Increased in dehydration and renal insufficiency. No changes have been linked to PAH exposure.	Latimer et al. 2003
glucose	Low	Stable or decreased	There is evidence that PAH exposure decreases available blood glucose. However, laboratory error can occur from delay in separation of plasma/serum from cellular component of blood sample delay.	Alonso-Alvarez et al. 2007; Latimer et al. 2003

TABLE 2
A priori prioritization of hematological and biochemical values in birds potentially exposed to petroleum products.

VARIABLE	Priority	Predicted response to polycyclic aromatic hydrocarbon exposure	Biological Relevance	References
polychromasia	Low	Increased	Regenerative anemias result in increased variability in erythrocyte stain uptake (Wright's staining). This is a subjective classification.	Yamamoto et al. 1996
anisocytosis	Low	Increased	Regenerative anemias result in increased variability in erythrocyte size. This is a subjective classification.	Latimer et al., 2003
buffy coat	Low	Increased percentage	This estimate of the percentage of white blood cells in circulation may increase during inflammation and is directly correlated with white blood cell count.	Wardlaw and Levine 1983

Table 3
Number of individuals and the degree of visible oiling for American oystercatcher (AMOY), black skimmer (BLSK), brown pelican (BRPE), clapper rail (CLRA), great egrets (GREG), and seaside sparrow (SESP).

SPECIES	Reference					API				
	None	Trace	Light	Moderate	Heavy	None	Trace	Light	Moderate	Heavy
AMOY	21	0	0	0	0	11	31	1	0	0
BLSK	88	0	0	0	0	74	41	6	0	0
BRPE	48	0	0	0	0	68	23	0	0	0
CLRA	88	0	0	0	0	86	16	0	0	0
GREG	40	7	0	0	0	18	34	5	0	0
SESP	104	0	0	0	0	348	17	1	0	0

Table 4

Species differences for Heinz bodies, reticulocytes, haptoglobin, ferritin, packed cell volume (PCV), hemoglobin, mean corpuscular hemoglobin concentration (MCHC), total polycyclic aromatic hydrocarbons (PAH) mean cell volume (MCV), white blood cell count (WBC), heterophils, lymphocytes, basophils, eosinophils, monocytes, calcium (Ca), phosphorus (Phos), lactate dehydrogenase (LDH), creatinine phosphokinase (CPK), chloride (Cl), uric acid (UA), cholesterol (Chol), and aspartate aminotransferase (AST) found in American oystercatcher (AMOY), black skimmer (BLSK), brown pelican (BRPE), clapper rail (CLRA), great egrets (GREG) in reference areas and areas of potential impact (API).

Variable	Reference		API	
	Chi^2	p	Chi^2	p
Heinz Bodies	9.956	0.452	2.63	0.019
Reticulocytes	25.143	0.003	14.023	<0.001
Haptoglobin	12.604	0.306	3.616	0.006
Ferritin	22.673	0.002	14.691	0.000
PCV	40.154	<0.001	46.74	<0.001
Hemoglobin	33.008	<0.001	27.776	<0.001
MCHC	4.594	0.094	6.401	0.204
Total solids	15.397	<0.001	24.385	0.002
Total PAH	8.921	0.337	3.376	0.030
MCV	24.327	0.012	11.031	<0.001
WBC	66.661	<0.001	40.287	<0.001
Heterophils	37.138	<0.001	35.382	<0.001
Lymphocytes	41.216	<0.001	37.349	<0.001
Basophils	57.399	<0.001	44.66	<0.001
Eosinophils	129.788	<0.001	67.217	<0.001
Monocytes	57.03	<0.001	61.493	<0.001
Ca	34.583	<0.001	67.334	<0.001
Phos	16.463	0.001	16.221	<0.001
LDH	39.911	<0.001	21.961	<0.001
CPK	29.031	<0.001	22.954	<0.001
Cl	7.716	<0.001	26.762	0.052
UA	33.285	<0.001	67.77	<0.001
Chol	61.777	<0.001	62.929	<0.001
AST	116.071	<0.001	85.119	<0.001

Table 5

Number of erythrocytes containing Heinz bodies/1000 erythrocytes found in American oystercatcher (AMOY), black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference, areas of potential impact (API) with no visible oil, and API with visible oil. Clapper rails were excluded from this table as no Heinz bodies were identified in this species. Seaside sparrows were excluded because no Heinz body data were quantified for this species.

SPECIES	Classification	n	mean	SE
AMOY	Reference	10	0.00	0.00
	API not visibly oiled	7	6.43	6.43
	API oiled	21	6.81	2.61
BLSK	Reference	57	0.00	0.00
	API not visible oiled	25	7.76	4.68
	API oiled	26	12.58	4.63
BRPE	Reference	35	0.00	0.00
	API not visible oiled	28	0.96	0.67
	API oiled	20	2.30	1.30
GREG	Reference	45	0.09	0.09
	API not visible oiled	11	2.73	2.27
	API oiled	31	8.68	3.10

TABLE 6

Kruskal-Wallis and SAS Multtest post hoc comparison results for the number of erythrocytes containing Heinz bodies/1000 erythrocytes found in American oystercatcher (AMOY), black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference areas, areas of potential impact (API) with no visible oil on the bird, and API with visible oil on the bird (oiled). Asterisk represents significance ($p \leq 0.01$) and ** represents marginal significance ($0.05 \geq p > 0.01$). N/A indicates not applicable because of insignificant Chi^2 result. Clapper rails were excluded from this table, as no Heinz bodies were identified in this species. Seaside sparrows were excluded from this table because no Heinz body data were quantified for this species.

SPECIES	n	Chi^2	p	Contrast	Bootstrap p-value
AMOY	37	2.64	0.267	reference vs no visible oil	N/A
				reference vs oiled	N/A
				no visible oil vs oiled	N/A
BLSK	108	19.37	<0.001*	reference vs no visible oil	0.865
				reference vs oiled	<0.001*
				no visible oil vs oiled	0.002*
BRPE	83	7.44	0.024**	reference vs no visible oil	0.201
				reference vs oiled	0.012**
				no visible oil vs oiled	0.522
GREG	87	11.81	0.003*	reference vs no visible oil	0.551
				reference vs oiled	0.003*
				no visible oil vs oiled	3.55

TABLE 7

Number of reticulocytes/1000 erythrocytes found in American oystercatcher (AMOY), black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference, areas of potential impact (API) with no visible oil, and API with visible oil. Clapper rails were excluded from this table because of the paucity of visibly-oiled birds from API sites with reticulocyte data. Seaside sparrows were excluded because no reticulocyte data were quantified for this species.

SPECIES	Classification	n	mean	SE
AMOY	Reference	10	44.00	2.28
	API no visible oil	7	54.43	3.28
	API oiled	21	61.67	2.57
BLSK	Reference	57	53.58	1.70
	API no visible oil	25	66.16	3.83
	API oiled	26	67.31	4.19
BRPE	Reference	35	44.51	1.55
	API no visible oil	28	60.14	2.48
	API oiled	20	65.60	3.42
GREG	Reference	45	53.60	2.50
	API no visible oil	11	57.27	7.34
	API oiled	31	68.13	4.00

TABLE 8

Kruskal-Wallis and SAS Multtest post hoc comparison results for the number of reticulocytes/1000 erythrocytes found in American oystercatcher (AMOY), black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference areas, areas of potential impact (API) with no visible oil on the bird, and API with visible oil on the bird (oiled). Asterisk represents significance ($p \leq 0.01$). Two asterisks represent marginal significance ($0.05 \geq p > 0.01$). Clapper rails were excluded from this table because of the paucity of oiled birds from API sites with reticulocyte data. Seaside sparrows were excluded because no reticulocyte data were quantified for this species.

SPECIES	n	Chi^2	p	Contrast	Bootstrap p-value
AMOY	38	15.03	<0.001*	reference vs no visible oil	0.057
				reference vs oiled	<0.001*
				no visible oil vs oiled	0.260
BLSK	108	11.33	0.004*	reference vs no visible oil	0.019**
				reference vs oiled	0.013**
				no visible oil vs oiled	0.865
BRPE	83	36.41	<0.001*	reference vs no visible oil	<0.001*
				reference vs oiled	<0.001*
				no visible oil vs oiled	0.317
GREG	87	9.31	0.010*	reference vs no visible oil	0.385
				reference vs oiled	0.004*
				no visible oil vs oiled	0.686

TABLE 9

Packed cell volume (PCV, percent packed cells) found in American oystercatcher (AMOY), black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference, areas of potential impact (API) with no visible oil, and API with visible oil. Clapper rails were excluded from this table because of the paucity of oiled birds from API sites with PCV data (n=2). Seaside sparrows were excluded because no PCV data were quantified for this species.

SPECIES	Classification	n	mean	SE
AMOY	Reference	21	47.38	0.76
	API no visible oil	6	42.33	1.23
	API oiled	23	41.91	1.17
BLSK	Reference	56	44.66	0.86
	API no visible oil	26	42.38	0.87
	API oiled	21	43.02	1.48
BRPE	Reference	36	50.22	0.89
	API no visible oil	25	41.86	0.67
	API oiled	20	43.28	0.84
GREG	Reference	44	46.05	0.57
	API no visible oil	12	35.20	1.77
	API oiled	33	37.30	0.79

TABLE 10

Kruskal-Wallis and SAS Multtest post hoc comparison results for packed cell volume (PCV) found in American oystercatcher (AMOY), black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference areas, areas of potential impact (API) with no visible oil on the bird, and API with visible oil on the bird (oiled). Asterisk represents significance ($p \leq 0.01$). N/A indicates not applicable because of insignificant Chi^2 result. Clapper rails were excluded from this table because of the paucity of oiled birds from API sites with PCV data (n=2). Seaside sparrows were excluded because no PCV data were quantified for this species.

SPECIES	n	Chi^2	p	Contrast	Bootstrap p-value
AMOY	50	18.88	<0.001*	reference vs no visible oil	0.005*
				reference vs oiled	<0.001*
				no visible oil vs oiled	0.998
BLSK	103	3.62	0.164	reference vs no visible oil	N/A
				reference vs oiled	N/A
				no visible oil vs oiled	N/A
BRPE	81	36.95	<0.001*	reference vs no visible oil	<0.001*
				reference vs oiled	<0.001*
				no visible oil vs oiled	0.455
GREG	89	54.43	<0.001*	reference vs no visible oil	<0.001*
				reference vs oiled	<0.001*
				no visible oil vs oiled	0.679

TABLE 11

Hemoglobin (Hb, g/dl) found in black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference sites, areas of potential impact (API) with no visible oil, and API with visible oil. Clapper rails were excluded from this table because of the paucity of oiled birds from API sites with Hb data (n=3). American oystercatchers were excluded from this table because no Hb data were collected in birds from reference areas. Seaside sparrows were excluded because no Hb data were quantified for this species.

SPECIES	Classification	n	mean	SE
BLSK	Reference	44	17.00	0.38
	API no visible oil	15	16.48	0.64
	API oiled	19	16.82	0.98
BRPE	Reference	33	17.42	0.47
	API no visible oil	20	15.64	0.25
	API oiled	18	15.63	0.38
GREG	Reference	44	15.94	0.38
	API no visible oil	12	13.48	0.73
	API oiled	33	13.93	0.53

TABLE 12

Kruskal-Wallis and SAS Multtest post hoc comparison results for hemoglobin (Hb) found in black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference areas, areas of potential impact (API) with no visible oil on the bird, and API with visible oil on the bird (oiled). Asterisk represents significance ($p \leq 0.01$). N/A indicates not applicable because of insignificant Chi^2 result. Clapper rails were excluded from this table because only one bird from API sites had Hb data. American oystercatchers were excluded from this table because no Hb data were collected in birds from reference areas. Seaside sparrows were excluded because no Hb data were quantified for this species.

SPECIES	n	Chi^2	p	Contrast	Bootstrap p-value
BLSK	78	0.26	0.876	reference vs no visible oil	N/A
				reference vs oiled	N/A
				no visible oil vs oiled	N/A
BRPE	71	12.65	0.002*	reference vs no visible oil	0.003*
				reference vs oiled	0.010*
				no visible oil vs oiled	0.977
GREG	89	18.99	<0.001*	reference vs no visible oil	0.003*
				reference vs oiled	<0.001*
				no visible oil vs oiled	0.989

TABLE 13

Mean corpuscular hemoglobin concentration (MCHC) found in black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference, areas of potential impact (API) with no visible oil, and API with visible oil. Clapper rails were excluded from this table because only one bird from API sites had MCHC data. American oystercatchers were excluded from this table because no MCHC data were collected in birds from reference areas. Seaside sparrows were excluded because no MCHC data were quantified for this species.

SPECIES	Classification	n	mean	SE
BLSK	Reference	43	38.16	1.25
	API no visible oil	15	39.07	2.36
	API oiled	17	39.43	2.70
BRPE	Reference	32	34.68	1.06
	API no visible oil	19	37.56	0.64
	API oiled	18	35.86	0.96
GREG	Reference	42	34.87	1.00
	API no visible oil	12	40.52	2.74
	API oiled	33	37.48	1.26

TABLE 14

Kruskal-Wallis and SAS Multtest post hoc comparison results for mean corpuscular hemoglobin concentration (MCHC) found in black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference areas, areas of potential impact (API) with no visible oil on the bird , and API with visible oil on the bird. N/A indicates not applicable because of insignificant Chi^2 result. Clapper rails were excluded from this table because only one bird from API sites had MCHC data. American oystercatchers were excluded from this table because no MCHC data were collected in birds from reference areas. Seaside sparrows were excluded because no MCHC data were quantified for this species.

SPECIES	n	Chi^2	p	Contrast	Bootstrap p-value
BLSK	75	0.26	0.878	reference vs no visible oil	N/A
				reference vs oiled	N/A
				no visible oil vs oiled	N/A
BRPE	69	4.06	0.134	reference vs no visible oil	N/A
				reference vs oiled	N/A
				no visible oil vs oiled	N/A
GREG	87	3.27	0.195	reference vs no visible oil	N/A
				reference vs oiled	N/A
				no visible oil vs oiled	N/A

TABLE 15

Red blood cell count (RBC, million cells/mm^3) found in black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference, areas of potential impact (API) with no visible oil, and API with visible oil. Clapper rails were excluded from this table because of the paucity of oiled birds from API sites with RBC data (n=3). American oystercatchers were excluded from this table because no RBC data were quantified for this species. Seaside sparrows were excluded because no RBC data were quantified for this species.

SPECIES	Classification	n	mean	SE
BLSK	Reference	59	2.72	0.03
	API no visible oil	17	2.55	0.11
	API oiled	17	2.64	0.07
BRPE	Reference	22	2.89	0.05
	API no visible oil	16	2.86	0.11
	API oiled	9	2.84	0.06
GREG	Reference	42	2.63	0.03
	API no visible oil	7	2.22	0.13
	API oiled	25	2.43	0.07

TABLE 16

Kruskal-Wallis and SAS Multtest post hoc comparison results for red blood cell count (RBC) found in black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference areas, areas of potential impact (API) with no visible oil on the bird, and API with visible oil on the bird (oiled). Asterisk represents significance ($p \leq 0.01$). N/A indicates not applicable because of insignificant Chi^2 result. Clapper rails were excluded from this table because of the paucity of oiled birds from API sites with RBC data (n=3). American oystercatchers were excluded from this table because no RBC data were collected for this species. Seaside sparrows were excluded because no RBC data were collected for this species.

SPECIES	n	Chi^2	p	Contrast	Bootstrap p-value
BLSK	93	0.98	0.613	reference vs no visible oil	N/A
				reference vs oiled	N/A
				no visible oil vs oiled	N/A
BRPE	47	1.16	0.561	reference vs no visible oil	N/A
				reference vs oiled	N/A
				no visible oil vs oiled	N/A
GREG	74	11.35	0.003*	reference vs no visible oil	0.006*
				reference vs oiled	0.053
				no visible oil vs oiled	0.231

TABLE 17

Mean cell volume (MCV, fL) found in black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference, areas of potential impact (API) with no visible oil, and API with visible oil. Clapper rails were excluded from this table because of the paucity of oiled birds from API sites with RBC data (n=3). American oystercatchers were excluded from this table because no MCV data were collected for this species. Seaside sparrows were excluded because no MCV data were collected for this species.

SPECIES	Classification	n	mean	SE
BLSK	Reference	47	162.79	2.91
	API no visible oil	13	169.39	11.87
	API oiled	11	154.89	7.56
BRPE	Reference	20	173.82	4.16
	API no visible oil	13	148.18	5.17
	API oiled	8	148.98	4.23
GREG	Reference	41	174.39	2.27
	API no visible oil	7	162.35	9.79
	API oiled	25	153.94	2.02

TABLE 18

Kruskal-Wallis and SAS Multtest post hoc comparison results for mean cell volume (MCV) found in black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference areas, areas of potential impact (API) with no visible oil on the bird, and API with visible oil on the bird (oiled). Asterisk represents significance ($p \leq 0.01$) and ** represents marginal significance ($0.05 \geq p > 0.01$). N/A indicates not applicable because of insignificant Chi^2 result. Clapper rails were excluded from this table because of the paucity of oiled birds from API sites with RBC data (n=3). American oystercatchers were excluded from this table because no MCV data were collected for this species. Seaside sparrows were excluded because no MCV data were collected for this species.

SPECIES	n	Chi^2	p	Contrast	Bootstrap p-value
BLSK	71	2.04	0.360	reference vs no visible oil	N/A
				reference vs oiled	N/A
				no visible oil vs oiled	N/A
BRPE	41	17.01	<0.001*	reference vs no visible oil	0.002*
				reference vs oiled	<0.001*
				no visible oil vs oiled	0.977
GREG	73	24.73	<0.001*	reference vs no visible oil	<0.035**
				reference vs oiled	<0.001*
				no visible oil vs oiled	0.231

TABLE 19

Haptoglobin (g/dl) found in American oystercatcher (AMOY), black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference, areas of potential impact (API) with no visible oil, and API with visible oil. Clapper rails were excluded from this table because of the small number of this species with ferritin data (n=8). Seaside sparrows were excluded from this table because no haptoglobin data were quantified for this species.

SPECIES	Classification	n	mean	SE
AMOY	Reference	17	0.25	0.02
	API no visible oil	5	0.32	0.06
	API oiled	13	0.23	0.01
BLSK	Reference	43	0.62	0.07
	API no visible oil	13	0.31	0.04
	API oiled	14	0.47	0.06
BRPE	Reference	33	0.59	0.11
	API no visible oil	21	0.52	0.07
	API oiled	19	0.48	0.08
GREG	Reference	48	0.63	0.05
	API no visible oil	13	0.54	0.08
	API oiled	28	0.61	0.06

TABLE 20

Kruskal-Wallis and SAS Multtest post hoc comparison results for haptoglobin found in American oystercatcher (AMOY), black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference areas, areas of potential impact (API) with no visible oil on the bird, and API with visible oil on the bird. ** represents marginal significance ($0.05 \leq p \geq 0.01$). N/A indicates not applicable because of insignificant Chi^2 result. Clapper rails were excluded from this table because of the small number of this species with ferritin data (n=8). Seaside sparrows were excluded because no haptoglobin data were quantified for this species.

SPECIES	n	Chi^2	p	Contrast	Bootstrap p-value
AMOY	35	2.28	0.319	reference vs no visible oil	N/A
				reference vs oiled	N/A
				no visible oil vs oiled	N/A
BLSK	70	8.54	0.014**	reference vs no visible oil	0.008*
				reference vs oiled	0.856
				no visible oil vs oiled	0.099
BRPE	73	0.54	0.762	reference vs no visible oil	N/A
				reference vs oiled	N/A
				no visible oil vs oiled	N/A
GREG	89	0.96	0.618	reference vs no visible oil	N/A
				reference vs oiled	N/A
				no visible oil vs oiled	N/A

Table 21

Ferritin concentration (ng/ml) found in black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference, areas of potential impact (API) with no visible oil, and API with visible oil. Clapper rails were excluded from this table because of the small number of this species with ferritin data (n=8). Seaside sparrows were excluded because no ferritin data were quantified for this species.

SPECIES	Classification	n	mean	SE
AMOY	Reference	17	40.70	4.16
	API no visible oil	5	37.01	5.73
	API oiled	13	27.44	4.82
BLSK	Reference	43	64.47	6.01
	API no visible oil	12	51.76	5.44
	API oiled	14	60.04	6.51
BRPE	Reference	31	50.28	4.47
	API no visible oil	19	62.83	5.83
	API oiled	17	63.42	4.67
GREG	Reference	42	69.58	3.87
	API no visible oil	11	28.55	5.84
	API oiled	26	38.76	4.82

TABLE 22

Kruskal-Wallis and SAS Multtest post hoc comparison results for ferritin found in black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference areas, areas of potential impact (API) with no visible oil on the bird, and API with visible oil on the bird (oiled). Asterisk represents significance ($p \leq 0.01$). N/A indicates not applicable because of insignificant Chi^2 result. Clapper rails were excluded from this table because of the small number of this species with ferritin data (n=8). Seaside sparrows were excluded because no ferritin data were quantified for this species.

SPECIES	n	Chi^2	p	Contrast	Bootstrap p-value
AMOY	35	5.24	0.073	reference vs no visible oil	N/A
				reference vs oiled	N/A
				no visible oil vs oiled	N/A
BLSK	69	1.13	0.568	reference vs no visible oil	N/A
				reference vs oiled	N/A
				no visible oil vs oiled	N/A
BRPE	67	3.38	0.184	reference vs no visible oil	N/A
				reference vs oiled	N/A
				no visible oil vs oiled	N/A
GREG	79	32.38	<0.001*	reference vs no visible oil	<0.001*
				reference vs oiled	<0.001*
				no visible oil vs oiled	0.376

TABLE 23.
Biochemistry parameters with significant effects from visible oiling including cholesterol and chloride in brown pelican (BRPE) and great egret (GREG) from reference, areas of potential impact (API) with no UV oiling, and API with trace, light, or moderate UV oiling. Asterisk represents significance ($p \leq 0.01$) from reference sites.

SPECIES	PARAMETER	Classification	n	Mean	SE
BRPE	**cholesterol**	Reference	24	202.67	9.14
	$Chi^2 = 24.59$	API no visible oil	19	149.48*	4.90
	$p < 0.001$	API visible oil	21	153.95*	4.61
GREG	**cholesterol**	Reference	37	203.03	4.34
	$Chi^2 = 53.44$	API no visible oil	10	126.10*	7.30
	$p < 0.001$	API visible oil	30	124.10*	4.10
GREG	**chloride**	Reference	35	101.26	0.82
	$Chi^2 = 41.12$	API no visible oil	11	109.91*	1.08
	$p < 0.001$	API visible oil	29	112.90*	2.06

TABLE 24
Numbers and degree of ultraviolet oiling in American oystercatcher (AMOY), black skimmer (BLSK), brown pelican (BRPE), clapper rail (CLRA), great egrets (GREG), and seaside sparrow (SESP).

SPECIES	Reference					API				
	None	Trace	Light	Moderate	Heavy	None	Trace	Light	Moderate	Heavy
AMOY	0	0	0	0	0	6	5	1	0	1
BLSK	87	0	0	0	0	28	73	17	3	0
BRPE	39	0	0	0	0	33	37	1	0	0
CLRA	29	1	0	0	0	19	71	10	3	1
GREG	20	27	1	0	0	8	41	4	1	0
SESP	55	0	0	0	0	240	143	15	1	0

TABLE 25

Number of erythrocytes containing Heinz bodies/1000 erythrocytes found in black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference, areas of potential impact (API) with no UV oiling, and API with trace, light, or moderate UV oiling. N/A indicates not applicable because n=1. Clapper rails were excluded from this table as no Heinz bodies were identified in this species. American oystercatchers were excluded from this table because no UV oiling data were collected from reference sites. Seaside sparrows were excluded because no Heinz body data were quantified for this species.

SPECIES	Classification	n	mean	SE
BLSK	Reference	57	0.00	0.00
	API no UV oil	7	15.71	15.71
	API trace	35	4.37	1.75
	API light	6	29.67	12.33
	API moderate	3	26.67	26.67
BRPE	Reference	32	0.00	0.00
	API no UV oil	16	0.00	0.00
	API trace	28	1.64	0.94
GREG	Reference	45	0.09	0.09
	API no UV oil	7	3.57	3.57
	API trace	35	3.49	1.26
	API light	3	53.00	6.66
	API moderate	1	35.00	N/A

TABLE 26

Kruskal-Wallis and SAS Multtest post hoc comparison results for the number of erythrocytes containing Heinz bodies/1000 erythrocytes found in black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) reference areas, and areas of potential impact (API) with no UV oiling, and API with trace, light, or moderate UV oiling. Asterisk represents significance ($p \leq 0.01$). Double asterisk indicates marginal significance ($0.05 \geq p > 0.01$). Clapper rails were excluded from this table, as no Heinz bodies were identified in this species. Seaside sparrows were excluded from this table because no Heinz body data were collected for this species. Only significant results are included in this table.

SPECIES	n	Chi^2	p	Contrast	Bootstrap p-value
BLSK	108	28.7	<0.001*	API light vs reference	<0.001*
				API trace vs reference	0.005*
				API light vs no UV oil	0.037*
				API light vs API trace	<0.001*
BRPE	76	7.14	0.028**	API trace vs reference	0.029**
GREG	91	35.25	<0.001*	API light vs reference	<0.001*
				API light vs no UV oil	0.003
				API light vs API trace	<0.001*

TABLE 27

Number of reticulocytes/1000 erythrocytes found black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference, areas of potential impact (API) with no UV oiling, and API with trace, light, or moderate UV oiling. N/A indicates not applicable because n=1. American oystercatchers were excluded from this table because no UV oiling data were collected from reference sites. Clapper rails were excluded from this table due to small sample size. Seaside sparrows were excluded because no Heinz body data were collected for this species.

SPECIES	Classification	n	mean	SE
BLSK	Reference	57	53.58	1.70
	API no UV oil	7	60.43	8.86
	API trace	35	65.29	2.80
	API light	6	79.83	9.59
	API moderate	3	72.33	23.85
BRPE	Reference	32	44.28	1.69
	API no UV oil	16	58.69	3.62
	API trace	28	63.71	2.63
GREG	Reference	45	54.26	2.53
	API no UV oil	7	58.43	6.48
	API trace	35	68.63	4.58
	API light	3	94.00	26.66
	API moderate	1	86.00	NA

TABLE 28

Kruskal-Wallis and SAS Multtest post hoc comparison results for the number of reticulocytes/1000 erythrocytes found in black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) reference areas, and areas of potential impact (API) with no UV oiling, and API with trace, light, or moderate UV oiling. Asterisk represents significance ($p \leq 0.01$). Double asterisk indicates marginal significance ($0.05 \geq p > 0.01$). Clapper rails were excluded from this table due to small sample size. Seaside sparrows were excluded from this table because no reticulocyte data were collected for this species. Only significant results are included in this table.

SPECIES	N	Chi^2	p	Contrast	Bootstrap p-value
BLSK	108	15.2	0.004*	API light vs reference	0.015**
				API trace vs reference	0.010*
BRPE	76	32.06	<0.001*	API trace vs reference	<0.001*
GREG	91	14.07	0.007*	API light vs reference	0.017**
				API trace vs reference	0.013**

TABLE 29

Packed cell volume (PCV) in black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference, areas of potential impact (API) with no UV oiling, and API with trace, light, or moderate UV oiling. N/A indicates not applicable because n=1. American oystercatchers were excluded from this table because no UV oiling data were collected from reference sites. Clapper rails were excluded from this table due to small sample size. Seaside sparrows were excluded because no PCV were quantified for this species.

SPECIES	Classification	n	mean	SE
BLSK	Reference	56	44.66	0.86
	API no UV oil	7	44.23	0.64
	API trace	30	41.83	1.00
	API light	7	43.86	2.09
	API moderate	3	44.57	6.74
BRPE	Reference	33	50.33	0.97
	API no UV oil	13	42.55	0.84
	API trace	27	43.02	0.67
GREG	Reference	45	45.84	0.59
	API no UV oil	7	38.14	2.19
	API trace	40	36.82	0.80
	API light	3	36.1	0.36
	API moderate	1	37.5	N/A

TABLE 30

Kruskal-Wallis and SAS Multtest post hoc comparison results for packed cell volume (PCV) in black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) reference areas, and areas of potential impact (API) with no UV oiling, and API with trace, light, or moderate UV oiling. Asterisk represents significance ($p \leq 0.01$). Clapper rails were excluded from this table due to small sample size. Seaside sparrows were excluded because no PCV were quantified for this species.

SPECIES	n	Chi^2	p	Contrast	Bootstrap p-value
BLSK	103	3.89	0.422	N/A	N/A
BRPE	73	30.18	<0.001*	API no UV oil vs reference	<0.001*
				API trace vs reference	<0.001*
GREG	96	55.26	<0.001*	API no UV oil vs reference	0.001*
				API trace vs reference	<0.001*
				API light vs reference	0.002*
				API light vs API trace	<0.001*

TABLE 31

Hemoglobin (Hb) in black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference, areas of potential impact (API) with no UV oiling, and API with trace, light, or moderate UV oiling. N/A indicates not applicable because n=1. American oystercatchers were excluded from this table because no UV oiling data were collected from reference sites. Clapper rails were excluded from this table due to small sample size. Seaside sparrows were excluded because no Hb data were collected for this species.

SPECIES	Classification	n	mean	SE
BLSK	Reference	44	17.00	0.38
	API no UV oil	4	16.55	0.64
	API trace	23	16.54	0.79
	API light	4	14.83	1.03
	API moderate	3	20.30	2.18
BRPE	Reference	31	17.27	0.46
	API no UV oil	11	15.43	0.38
	API trace	24	15.89	0.30
GREG	Reference	45	15.86	0.38
	API no UV oil	7	13.56	0.67
	API trace	40	13.61	0.50
	API light	3	13.73	0.33
	API moderate	1	13.10	N/A

TABLE 32

Kruskal-Wallis and SAS Multtest post hoc comparison results for hemoglobin (Hb) concentration found in black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) reference areas, and areas of potential impact (API) with no UV oiling, and API with trace, light, or moderate UV oiling. Asterisk represents significance ($p \leq 0.01$). Double asterisk indicates marginal significance ($0.05 \geq p > 0.01$). N/A indicates not applicable because of insignificant Chi^2 result. Clapper rails were excluded from this table due to small sample size. Seaside sparrows were excluded because no Hb data were collected for this species.

SPECIES	n	Chi^2	p	Contrast	Bootstrap p-value
BLSK	78	6.15	0.188	N/A	N/A
BRPE	66	10.07	0.006*	API no UV oil vs reference	0.029**
				API trace vs reference	0.018**
GREG	96	21.04	<0.001*	API trace vs reference	<0.001*

TABLE 33

Mean corpuscular hemoglobin concentration (MCHC) found black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference, areas of potential impact (API) with no UV oiling, and API with trace, light, or moderate UV oiling. N/A indicates not applicable because n=1. American oystercatchers were excluded from this table because no UV oiling data were collected from reference sites. Clapper rails were excluded from this table due to small sample size. Seaside sparrows were excluded because no MCHC data were collected for this species.

SPECIES	Chi^2	p value	Classification	n	mean	SE
BLSK	3.78	0.437	Reference	43	38.16	1.25
			API no UV oil	4	37.77	1.41
			API trace	21	39.31	2.37
			API light	4	34.14	1.35
			API moderate	3	47.74	8.90
BRPE	4.06	0.131	Reference	30	2.89	1.06
			API no UV oil	10	2.82	1.07
			API trace	23	2.89	0.79
GREG	3.20	0.523	Reference	44	34.89	0.97
			API no UV oil	7	36.10	2.54
			API trace	40	37.25	1.34
			API light	3	38.04	0.71
			API moderate	1	34.93	N/A

TABLE 34

Red blood cell count (RBC) found black skimmer (BLSK), brown pelican (BRPE), clapper rail (CPRL), and great egret (GREG) from reference, areas of potential impact (API) with no UV oiling, and API with trace, light, or moderate UV oiling. N/A indicates not applicable because n=1. American oystercatchers were excluded from this table because no UV oiling data were collected from reference sites. Seaside sparrows were excluded because no RBC data were quantified for this species.

SPECIES	Classification	n	mean	SE
BLSK	Reference	59	2.71	0.03
	API no UV oil	3	2.12	0.35
	API trace	25	2.64	0.08
	API light	4	2.69	0.05
	API moderate	2	2.61	0.03
BRPE	Reference	21	2.89	0.05
	API no UV oil	11	2.82	0.14
	API trace	12	2.89	0.07
CLRA	Reference	7	2.15	0.13
	API no UV oil	1	2.47	N/A
	API trace	14	2.25	0.10
	API light	3	2.31	0.20
	API moderate	1	2.46	N/A
GREG	Reference	43	2.63	0.03
	API no UV oil	5	2.20	0.18
	API trace	28	2.46	0.07
	API light	3	2.25	0.14
	API moderate	1	2.48	N/A

TABLE 35

Kruskal-Wallis and SAS Multtest post hoc comparison results for red blood cell counts (RBC) found in black skimmer (BLSK), brown pelican (BRPE), clapper rail (CPRL), and great egret (GREG) reference areas, and areas of potential impact (API) with no UV oiling, and API with trace, light, or moderate UV oiling. Double asterisk indicates marginal significance ($0.05 \geq p > 0.01$). N/A indicates not applicable because of insignificant Chi^2 result. American oystercatchers were excluded from this table because no UV oiling data were collected from reference sites. Seaside sparrows were excluded because no RBC data were quantified for this species.

SPECIES	n	Chi^2	p	Contrast	Bootstrap p-value
BLSK	93	5.28	0.259	N/A	N/A
BRPE	44	0.34	0.843	N/A	N/A
CPRL	26	1.18	0.882	N/A	N/A
GREG	80	12.79	0.012**	API no UV oil vs reference	0.050**
				API light vs reference	0.043**

TABLE 36

Mean cell volume (MCV) found black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference, areas of potential impact (API) with no UV oiling, and API with trace, light, or moderate UV oiling. N/A indicates not applicable because n=1. American oystercatchers were excluded from this table because no UV oiling data were collected from reference sites. Clapper rails were excluded from this table due to small sample size. Seaside sparrows were excluded because no PCV were quantified for this species.

SPECIES	Classification	n	mean	SE
BLSK	Reference	47	162.79	2.91
	API no UV oil	3	216.05	38.91
	API trace	15	152.46	6.00
	API light	4	170.03	12.57
	API moderate	2	145.35	5.43
BRPE	Reference	19	173.74	4.39
	API no UV oil	8	152.21	7.42
	API trace	11	145.79	3.93
GREG	Reference	42	173.84	2.29
	API no UV oil	5	170.63	11.24
	API trace	28	152.91	1.66
	API light	3	162.25	12.33
	API moderate	1	151.21	N/A

TABLE 37

Kruskal-Wallis and SAS Multtest post hoc comparison results for mean cell volume (MCV) in black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) in reference areas, and areas of potential impact (API) with no UV oiling, and API with trace, light, or moderate UV oiling. Asterisk represents significance ($p \leq 0.01$). Double asterisk indicates marginal significance ($0.05 \geq p > 0.01$). Clapper rails were excluded from this table due to small sample size. Seaside sparrows were excluded because no Hb data were collected for this species.

SPECIES	n	Chi^2	p	Contrast	Bootstrap p-value
BLSK	71	10.25	0.037**	API no UV oil vs trace	0.047**
BRPE	38	15.17	<0.001*	API trace vs reference	0.029**
GREG	79	28.03	<0.001*	API trace vs reference	<0.001*

TABLE 38

Haptoglobin concentration found black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference, areas of potential impact (API) with no UV oiling, and API with trace, light, or moderate UV oiling. N/A indicates not applicable because n=1. American oystercatchers were excluded from this table because no UV oiling data were collected from reference sites. Clapper rails were excluded from this table due to small sample size. Seaside sparrows were excluded because haptoglobin data were collected for this species.

SPECIES	Chi^2	p value	Classification	n	mean	SE
BLSK	5.13	0.274	Reference	43	0.62	0.07
			API no UV oil	4	0.36	0.13
			API trace	17	0.40	0.05
			API light	4	0.34	0.06
			API moderate	2	0.46	0.18
BRPE	4.06	0.131	Reference	31	0.59	0.12
			API no UV oil	13	0.48	0.09
			API trace	25	0.48	0.06
GREG	3.20	0.523	Reference	43	0.64	0.05
			API no UV oil	6	0.65	0.12
			API trace	35	0.58	0.05
			API light	2	0.36	0.18
			API moderate	1	0.53	N/A

Table 39.

Ferritin concentration found black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) from reference areas, and areas of potential impact (API) with no UV oiling, and API with trace, light, or moderate UV oiling. N/A indicates not applicable because n=1. American oystercatchers were excluded from this table because no UV oiling data were collected from reference sites. Clapper rails were excluded from this table due to small sample size. Seaside sparrows were excluded because no ferritin data were quantified for this species.

SPECIES	Classification	n	mean	SE
BLSK	Reference	43	64.47	6.01
	API no UV oil	4	63.03	9.96
	API trace	16	57.49	5.79
	API light	4	45.06	7.54
	API moderate	2	54.73	26.75
BRPE	Reference	29	50.68	4.94
	API no UV oil	11	51.72	7.19
	API trace	23	62.76	3.81
GREG	Reference	43	69.69	3.78
	API no UV oil	6	31.84	9.26
	API trace	33	38.16	4.12
	API light	2	33.89	15.92
	API moderate	1	34.20	N/A

TABLE 40.

Kruskal-Wallis and SAS Multtest post hoc comparison results for the ferritin concentration in black skimmer (BLSK), brown pelican (BRPE), and great egret (GREG) reference areas, and areas of potential impact (API) with no UV oiling, and API with trace, light, or moderate UV oiling. Asterisk represents significance ($p \leq 0.01$). Double asterisk indicates marginal significance ($0.05 \geq p > 0.01$). N/A indicates not applicable because of insignificant Chi^2 result. American oystercatchers were excluded from this table because no UV oiling data were collected from reference sites. Clapper rails were excluded from this table due to small sample size. Seaside sparrows were excluded because no ferritin data were quantified for this species.

SPECIES	n	Chi^2	p	Contrast	Bootstrap p-value
BLSK	69	1.82	0.768	N/A	N/A
BRPE	63	3.53	0.171	N/A	N/A
GREG	92	32.98	<0.001*	API no UV oil vs reference	<0.002*
				API trace vs reference	<0.001*

TABLE 41.

Biochemistry parameters with significant effects from UV oiling including cholesterol and chloride in brown pelican (BRPE) and great egret (GREG) from reference, areas of potential impact (API) with no UV oiling, and API with trace, light, or moderate UV oiling. Asterisk represents significance ($p \leq 0.01$) from reference sites. Double asterisk indicates marginal significance ($0.05 \geq p > 0.01$) from reference sites. N/A indicates not applicable because n =1.

SPECIES	PARAMETER	Classification	n	Mean	SE
BRPE	cholesterol $Chi^2 = 22.47$ $p < 0.001$	Reference	23	203.26	9.53
		API no UV oil	16	154.75*	8.83
		API trace	24	152.33*	4.09
		API light	1	170.00	N/A
GREG	cholesterol $Chi^2 = 52.23$ $p < 0.001$	Reference	37	201.08	4.66
		API no UV oil	3	136.00	8.29
		API trace*	32	123.31	4.15
		API light*	2	114.50	3.5
GREG	chloride $Chi^2 = 40.13$ $p < 0.001$	Reference	33	101.18	0.85
		API no UV oil	5	109.40**	0.93
		API trace*	30	112.97*	1.94
		API light	2	110.50	2.5

TABLE 42.
Number of birds with and without total polycyclic aromatic hydrocarbons (PAH) detected in blood.

SPECIES	PAH detected	PAH not detected
AMOY	4	29
BLSK	18	67
BRPE	9	74
CLRA	19	54
GREG	15	78

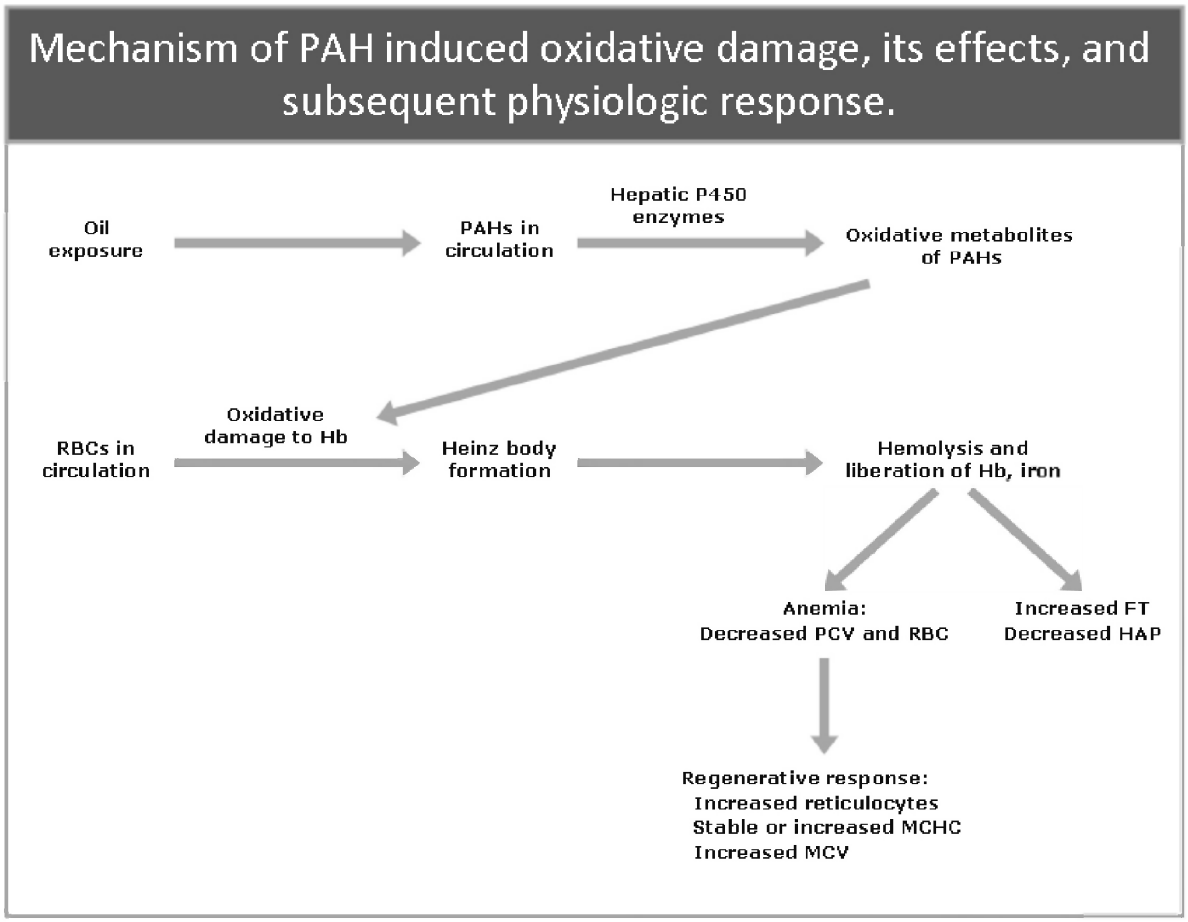

Figure 1. Conceptual diagram of the mechanism of polycyclic aromatic hydrocarbons (PAH) induced oxidative damage, its effects, and subsequent physiologic response in birds. Physiological variables include Heinz bodies, reticulocytes, hemoglobin (Hb), packed cell volume (PCV), red blood cell count (RBC), ferritin (FT), haptoglobin (HAP), mean corpuscular hemoglobin concentration (MCHC), and mean cell volume (MCV).

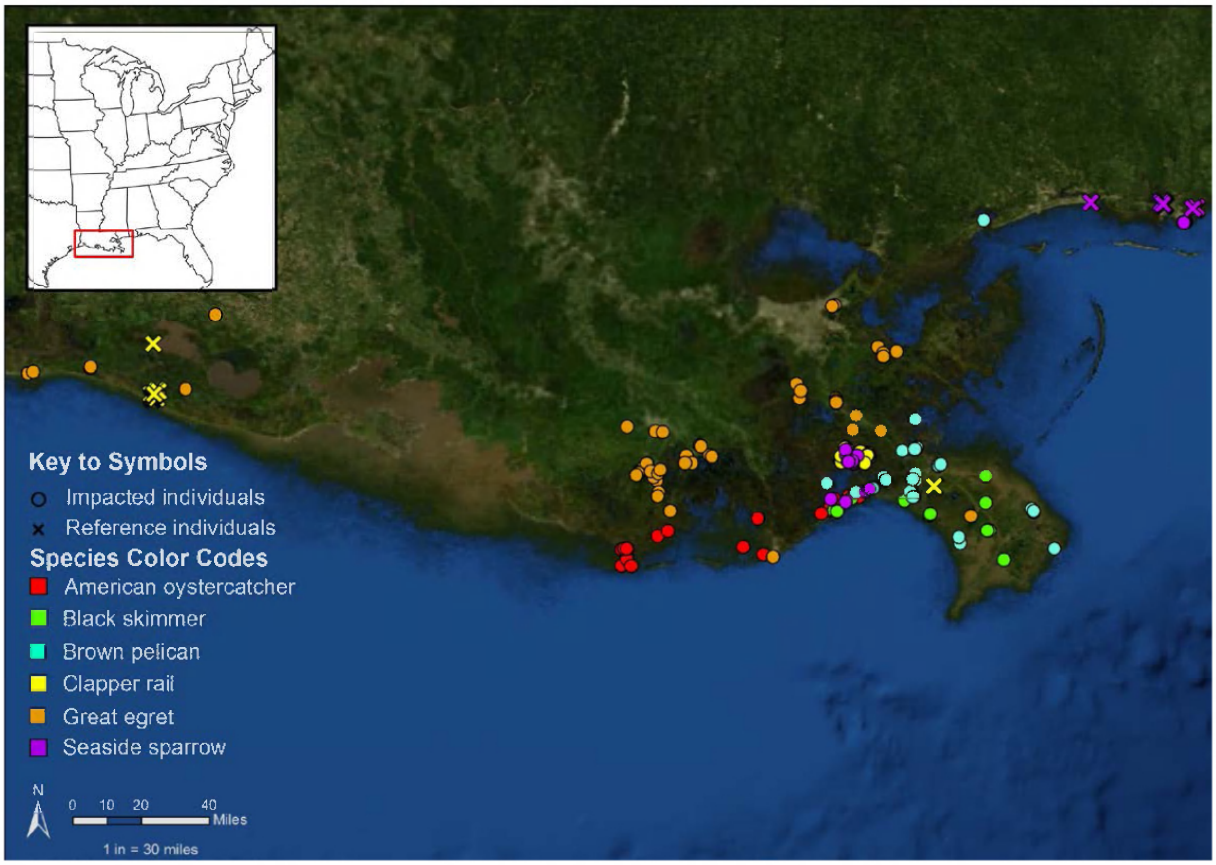

Figure 2. Area of potential impact capture locations in Louisiana, Mississippi and Alabama for American oystercatcher, black skimmer, brown pelican, and great egret. Also shown are reference capture locations and area of potential impact capture locations for clapper rail and seaside sparrow.

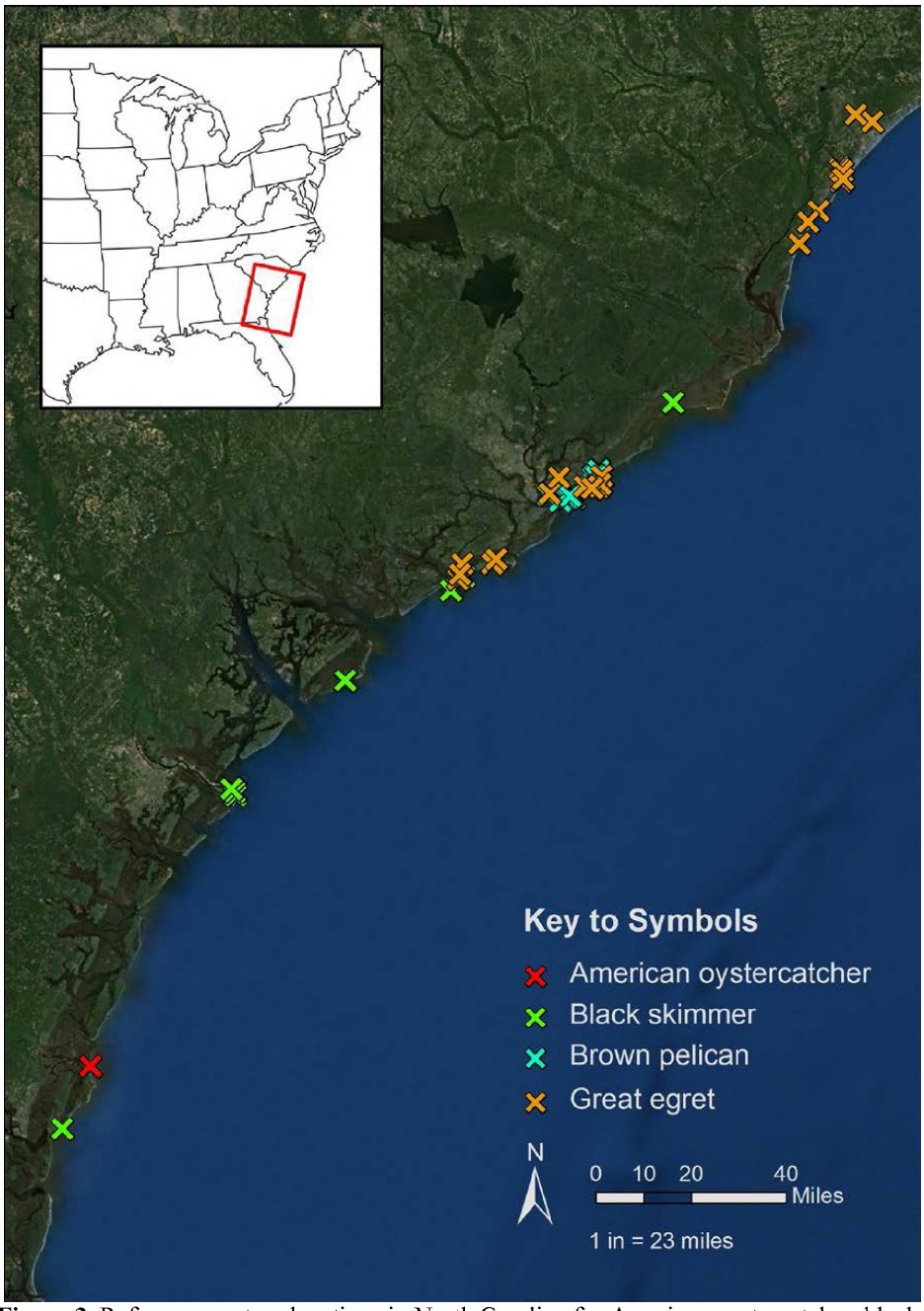

Figure 3. Reference capture locations in North Carolina for American oystercatcher, black skimmer, brown pelican, and great egret.

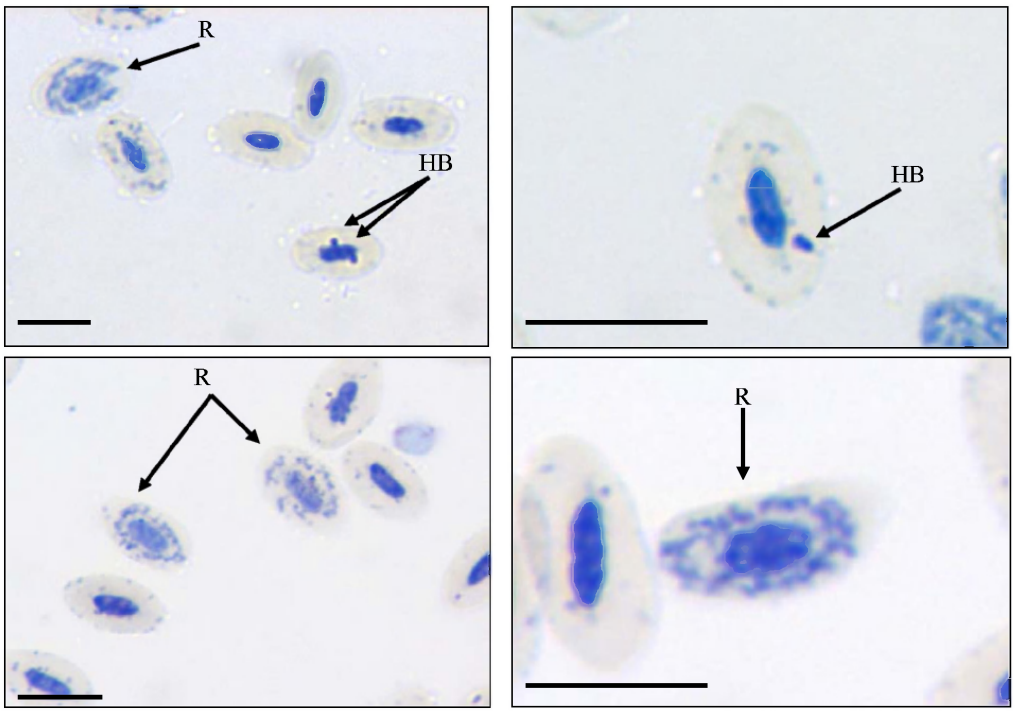

Figure 4. Air-dried new methylene blue stained brown pelican blood smears showing reticulocytes (R) and Heinz bodies (HB). Bar = 10 µm. Images from *in vivo* Heinz body induction.

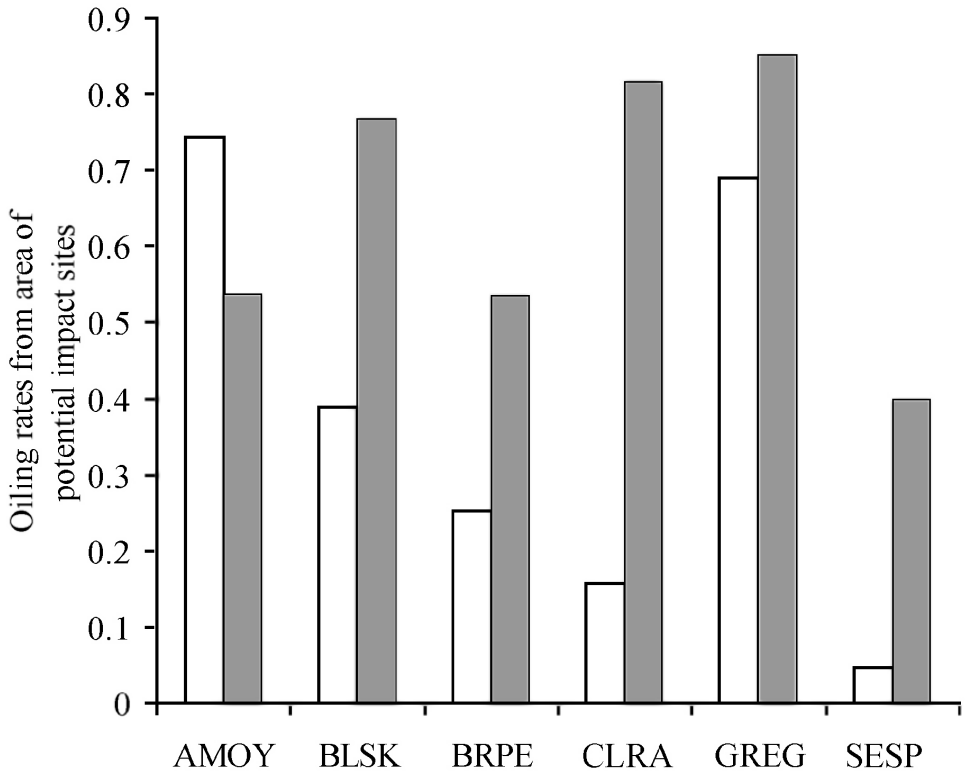

Figure 5: Visible oiling (non-shaded) and UV florescent oiling (shaded bars) rates for American oystercatcher (AMOY, n=75), black skimmer (BLSK, n=168), brown pelican (BRPE, n=114), clapper rail (CLRA, n=118) and great egret (GREG, n=96) and seaside sparrows (SESP, n=384) from areas of potential impact.

Note: the apparently anomalous result for AMOY is likely related to the relative sample size of individuals that were evaluated for UV oiling vs those with visible oiling assessment. Detailed interpretation is present within the results and discussion sections of this report.

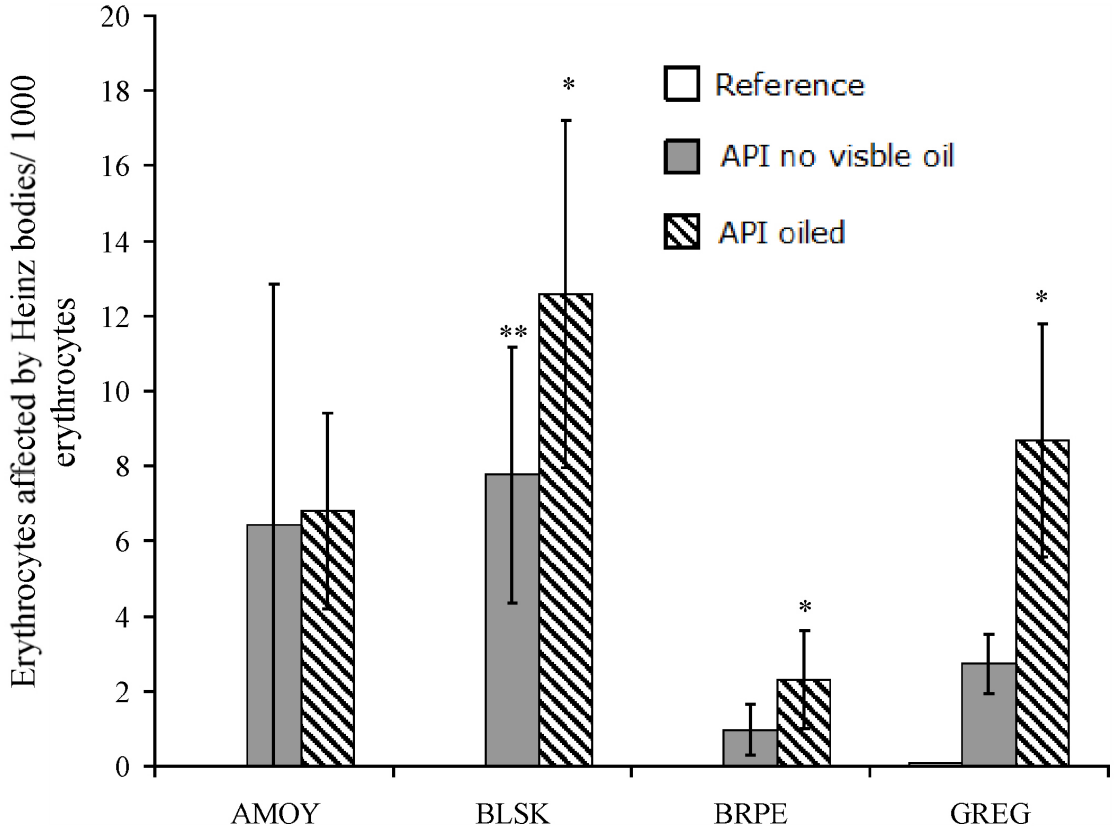

Figure 6: Number of erythrocytes containing Heinz bodies/1000 erythrocytes (Mean +/– SE) found in American oystercatcher (AMOY, n=38), black skimmer (BLSK, n=108), brown pelican (BRPE, n=83), and great egret (GREG, n=87) from reference (non-shaded bars), areas of potential impact with no visible oil (shaded bars), and areas of potential impact with visible oil (hashed bars). * indicates significant difference ($p<0.05$) from reference sites. ** indicates significant difference between API oiling categories. Clapper rails were excluded from this figure because of the paucity of birds from API with oil and because no Heinz bodies were identified in this species. Seaside sparrows were excluded because no Heinz body data were quantified for this species.

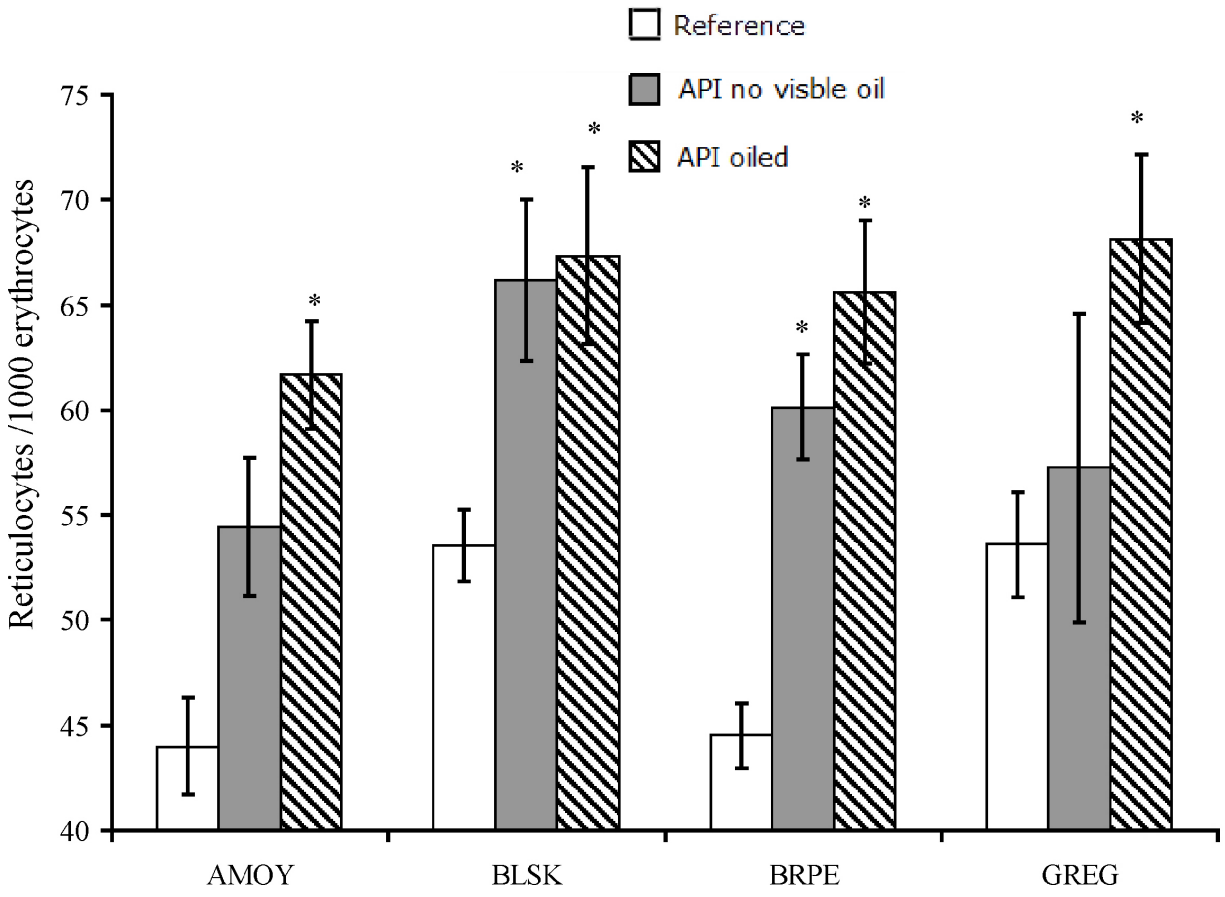

Figure 7: Number of reticulocytes/1000 erythrocytes (Mean +/– SE) found in American oystercatcher (AMOY, n=38), black skimmer (BLSK, n=108), brown pelican (BRPE, n=83), and great egret (GREG, n=87) from reference (non-shaded bars), areas of potential impact with no visible oil (shaded bars), and areas of potential impact with visible oil (hashed bars). Asterisks indicate significant difference ($p \leq 0.05$) from reference sites. Clapper rails were excluded from this figure because of the paucity of oiled birds from API sites with reticulocyte data. Seaside sparrows were excluded because no reticulocyte data were quantified for this species.

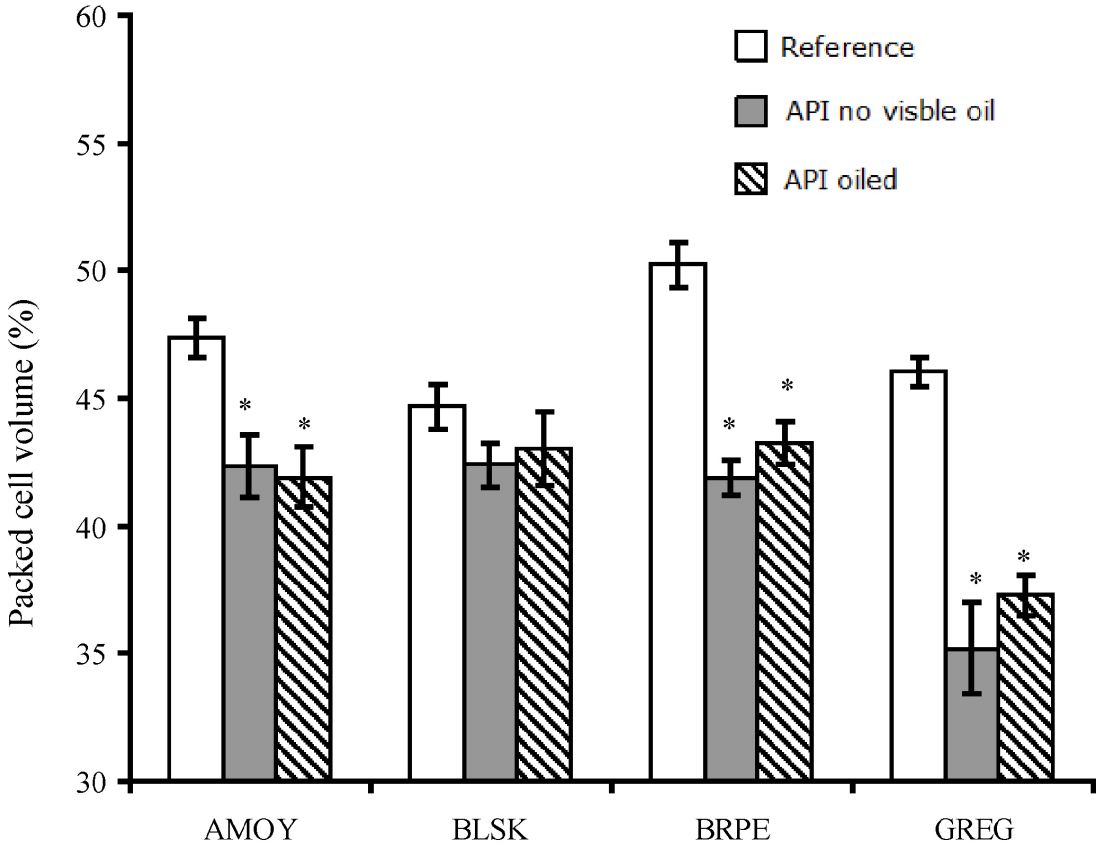

Figure 8: Packed cell volume (PCV) (Mean +/– SE) found in American oystercatcher (AMOY, n=50), black skimmer (BLSK, n=103), brown pelican (BRPE, n=81), and great egret (GREG, n=89) from reference (non-shaded bars), areas of potential impact with no visible oil (shaded bars), and areas of potential impact with visible oil (hashed bars). Asterisks indicate significant difference ($p \leq 0.05$) from reference sites. Clapper rails were excluded from this figure because of the paucity of oiled birds from API sites with PCV data. Seaside sparrows were excluded because no PCV data were quantified for this species.

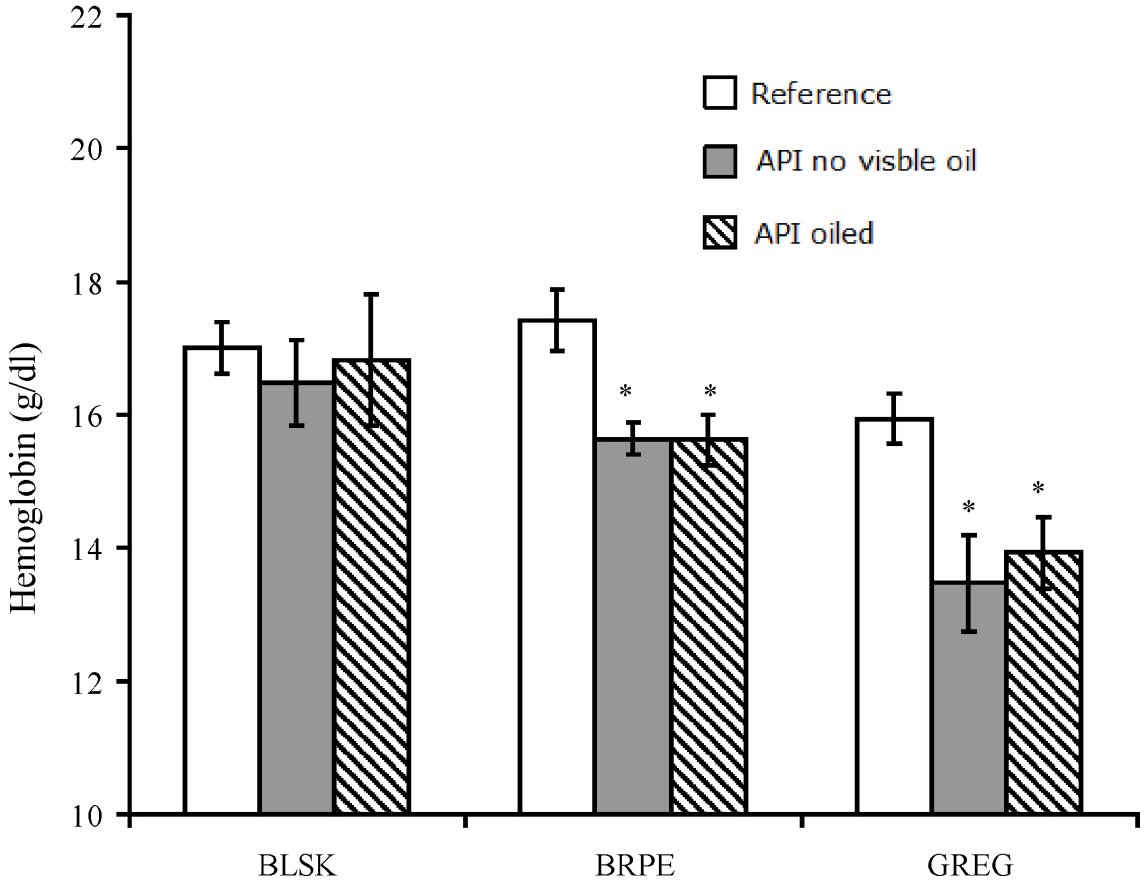

Figure 9: Hemoglobin (Hb) (Mean +/– SE) found in black skimmer (BLSK, n=78), brown pelican (BRPE, n=71), and great egret (GREG, n=89) from reference (non-shaded bars), areas of potential impact with no visible oil (shaded bars), and areas of potential impact with visible oil (hashed bars). Asterisks indicate significant difference ($p \leq 0.05$) from reference sites. Clapper rails were excluded from this table because of the paucity of oiled birds from API sites with Hb data (n=3). American oystercatchers were excluded from this table because no Hb data were collected for birds from reference areas. Seaside sparrows were excluded because no Hb data were quantified for this species.

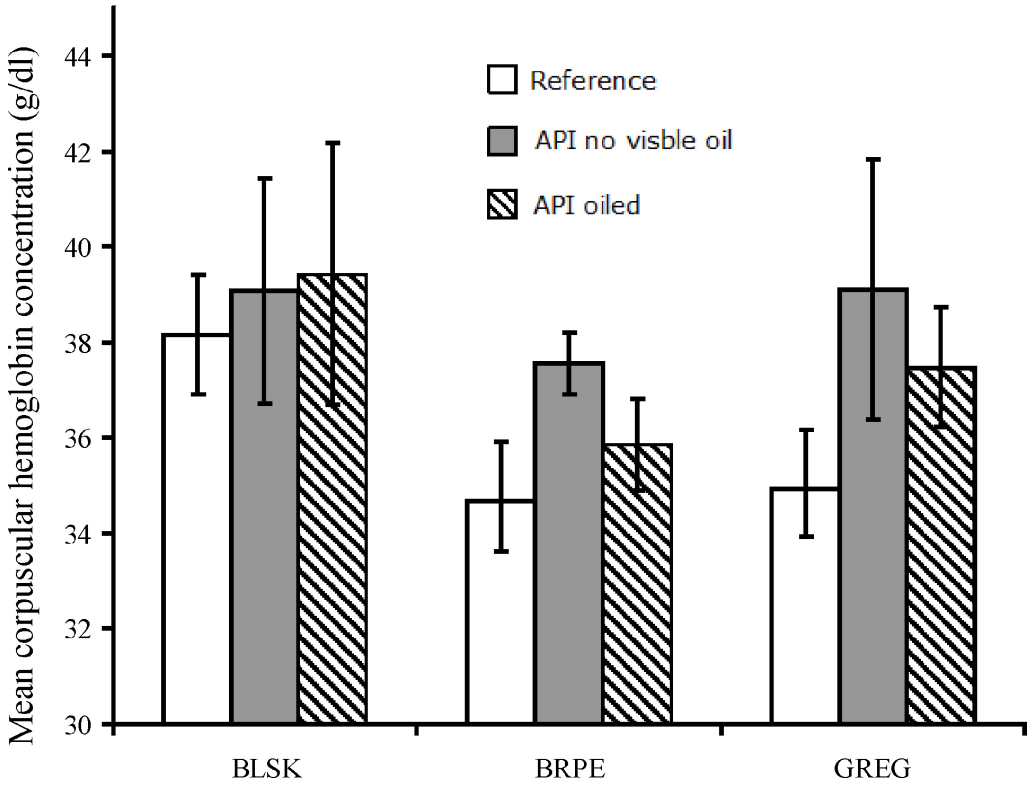

Figure 10: Mean corpuscular hemoglobin concentration (MCHC) (Mean +/– SE) found in black skimmer (BLSK, n=75), brown pelican (BRPE, n=69), and great egret (GREG, n=87) from reference (non-shaded bars), areas of potential impact with no visible oil (shaded bars), and areas of potential impact with visible oil (hashed bars). Clapper rails were excluded from this table because only one bird from API sites had MCHC data. American oystercatchers were excluded from this table because no data were collected for birds from reference areas. Seaside sparrows were excluded because no Hb data were quantified for this species

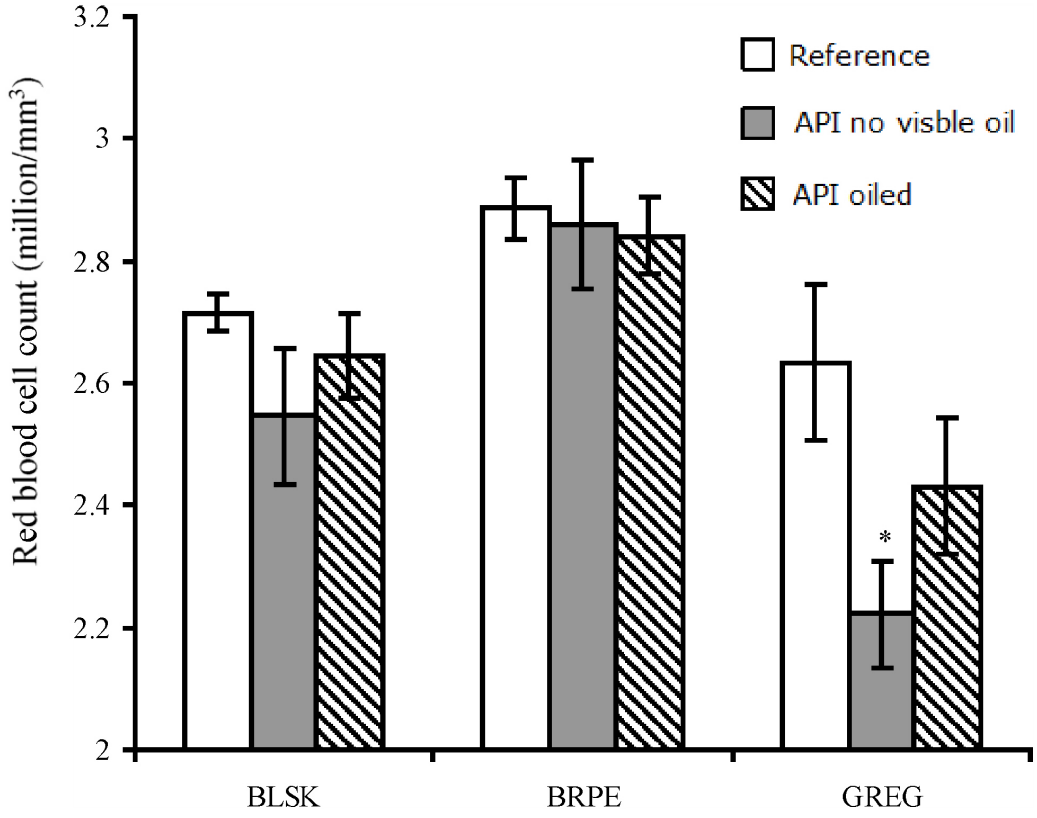

Figure 11: Red blood cell count (RBC) (Mean +/– SE) found in black skimmer (BLSK, n=93), brown pelican (BRPE, n=47), and great egret (GREG, n=74) from reference (non-shaded bars), areas of potential impact with no visible oil (shaded bars), and areas of potential impact with visible oil (hashed bars). Asterisks indicate significant difference ($p \leq 0.05$) from reference sites. Clapper rails were excluded from this table because of the paucity of oiled birds from API sites with RBC data (n=3). American oystercatchers were excluded from this table because no RBC data were quantified for this species. Seaside sparrows were excluded because no RBC data were quantified for this species.

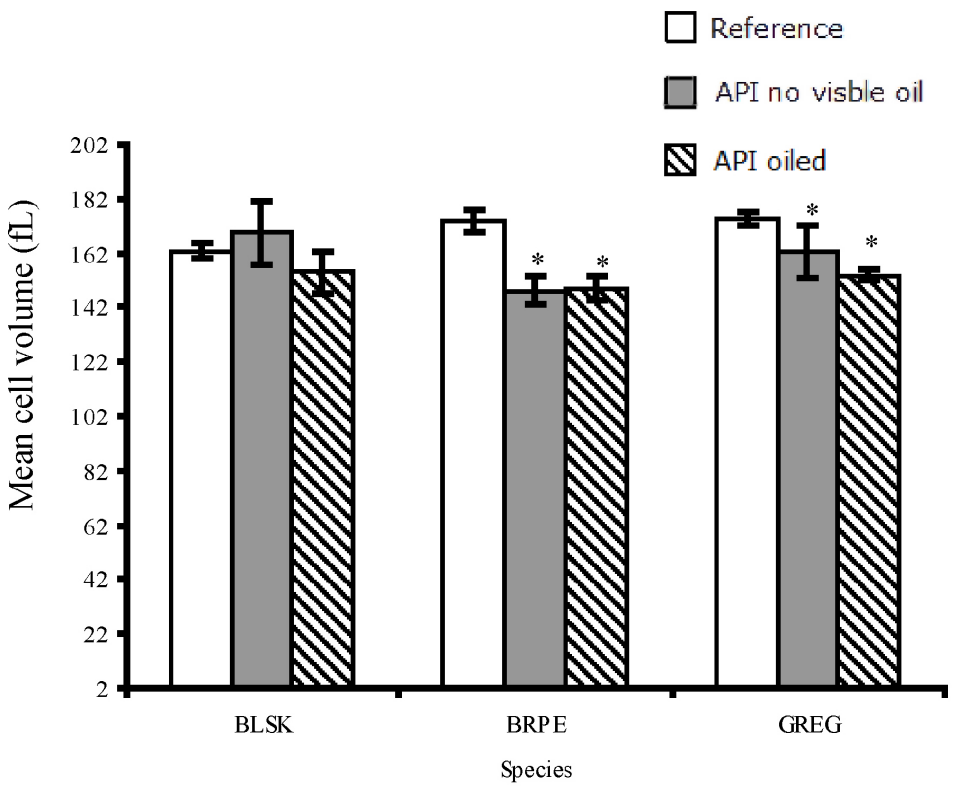

Figure 12: Mean cell volume (MCV) (Mean +/– SE) found in black skimmer (BLSK, n=71), brown pelican (BRPE, n=41), and great egret (GREG, n=73) from reference (non-shaded bars), areas of potential impact with no visible oil (shaded bars), and areas of potential impact with visible oil (hashed bars). Asterisks indicate significant difference ($p \leq 0.05$) from reference sites. Clapper rails were excluded from this table because of the paucity of oiled birds from API sites with MCV data (n=3). American oystercatchers were excluded from this table because no MCV data were quantified for this species. Seaside sparrows were excluded because no MCV data were quantified for this species.

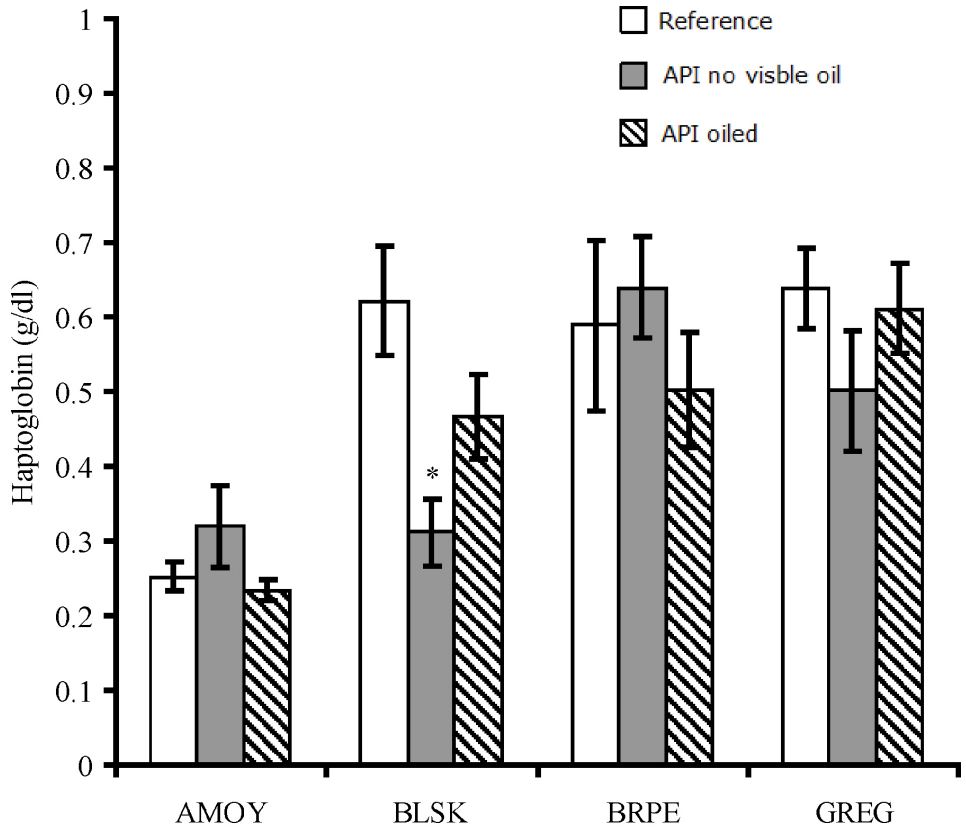

Figure 13: Haptoglobin concentration (Mean +/– SE) found in American oystercatcher (AMOY, n=35), black skimmer (BLSK, n=70), brown pelican (BRPE, n=73), and great egret (GREG, n=89) from reference (non-shaded bars), areas of potential impact with no visible oil (shaded bars), and areas of potential impact with visible oil (hashed bars). Asterisk indicates significant difference ($p<0.05$) from birds from reference site. Clapper rails were excluded from this table because of the small number of this species with haptoglobin data (n=8). Seaside sparrows were excluded from this table because no haptoglobin data were quantified for this species.

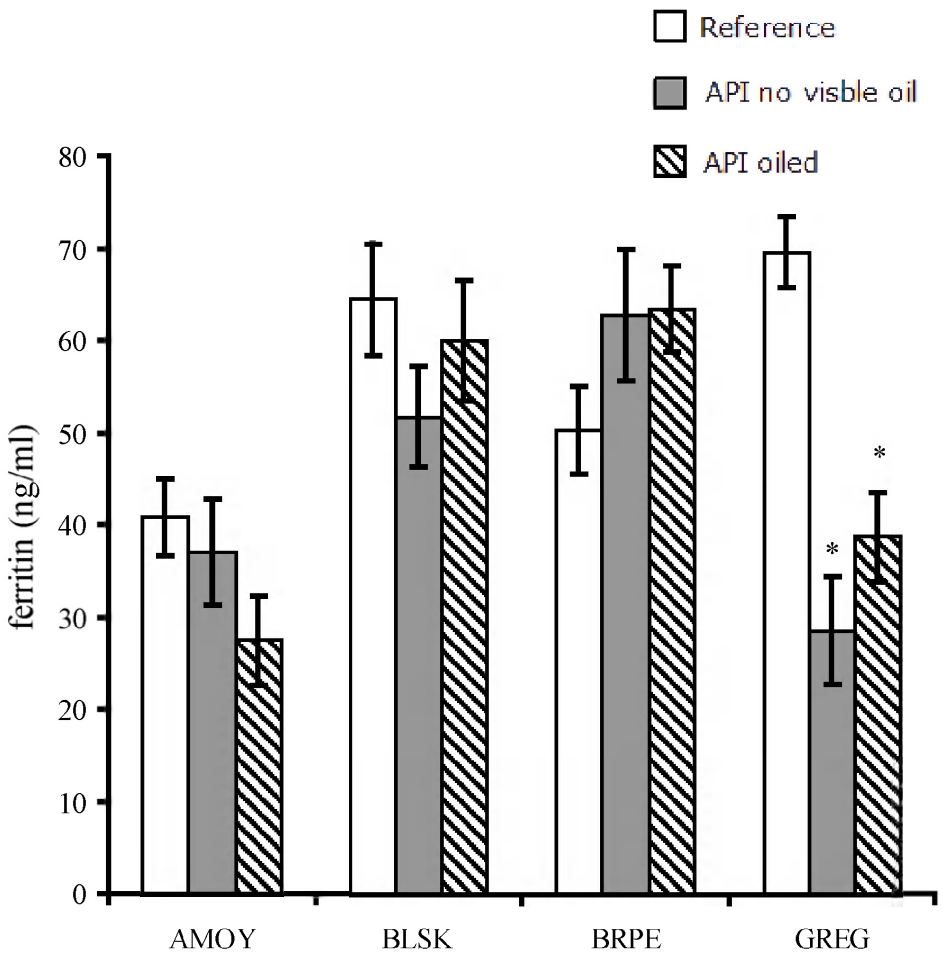

Figure 14: Ferritin concentration (Mean +/– SE) found in American oystercatcher (AMOY, n=35), black skimmer (BLSK, n=69), brown pelican (BRPE, n=67), and great egret (GREG, n=79) from reference (non-shaded bars), areas of potential impact with no visible oil (shaded bars), and areas of potential impact with visible oil (hashed bars). Asterisks indicate significant difference ($p<0.05$) from reference sites. Clapper rails were excluded from this table because of the small number of this species with ferritin data (n=8). Seaside sparrows were excluded from this table because no ferritin data were quantified for this species.

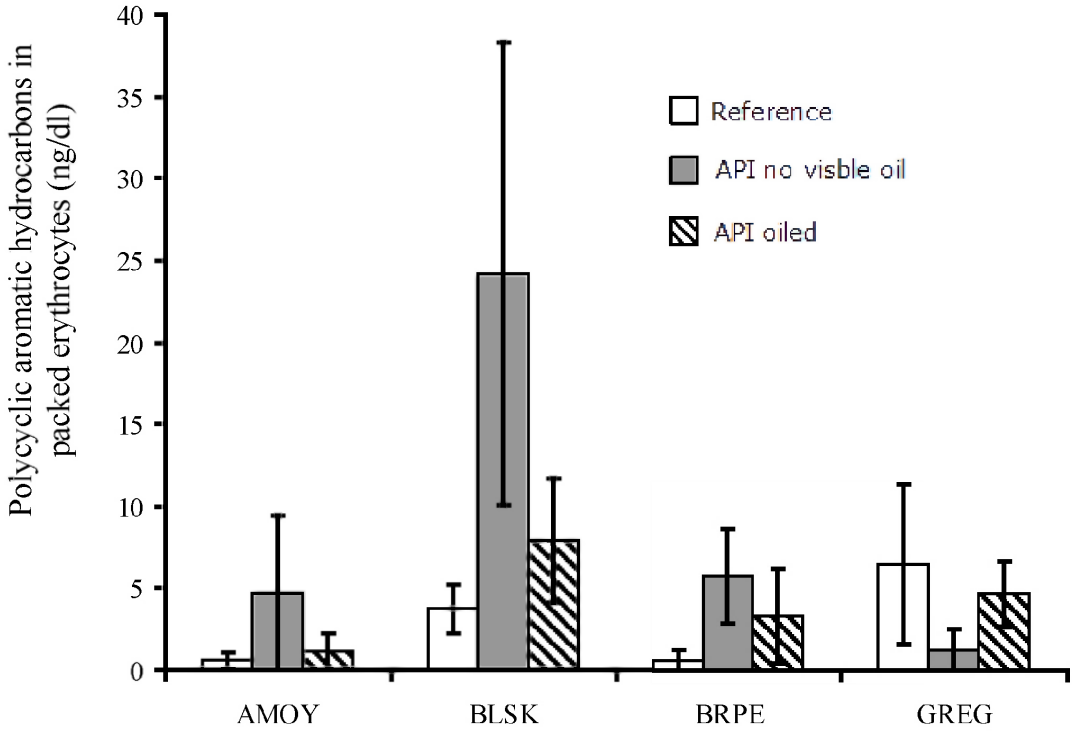

Figure 15: Total polycyclic aromatic hydrocarbons (PAH) in circulation (Mean +/– SE) found in American oystercatcher (AMOY, n=33), black skimmer (BLSK, n=84), brown pelican (BRPE, n=79), and great egret (GREG, n=92) from reference (non-shaded bars), areas of potential impact with no visible oil (shaded bars), and areas of potential impact with visible oil (hashed bars). Clapper rails were excluded from this table because of the small number of this species with PAH detected in the blood. Seaside sparrows were excluded from this table because no PAH data were quantified for this species.

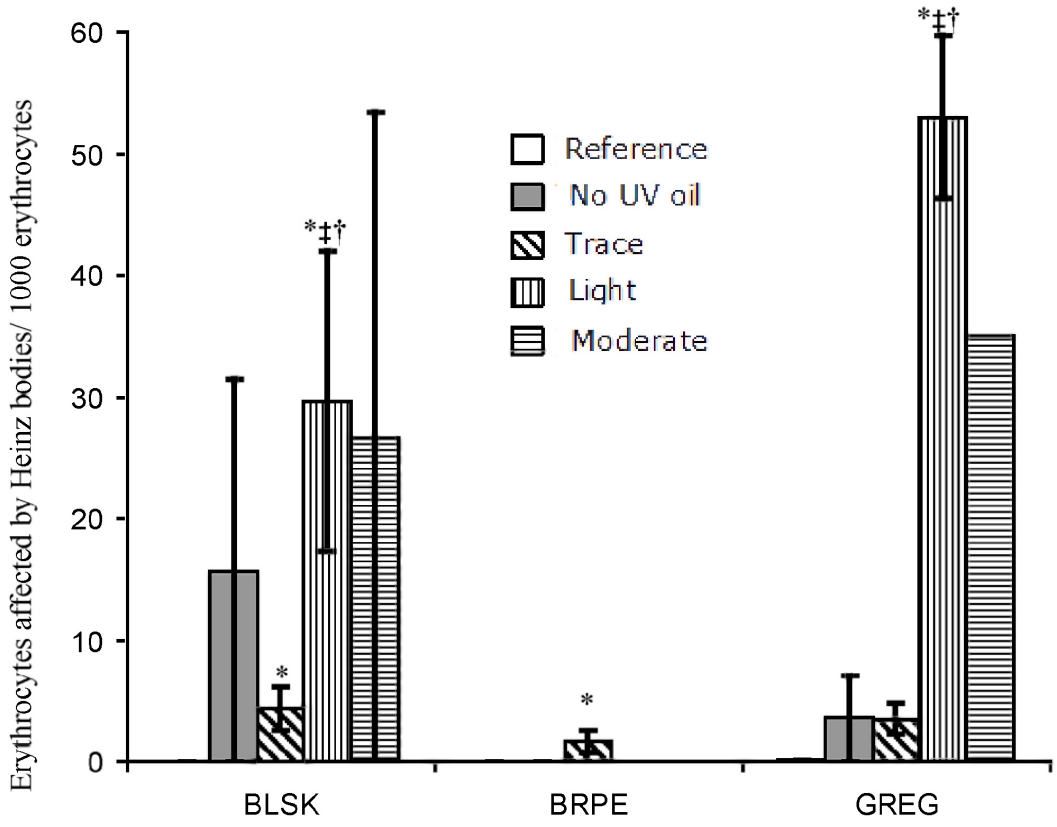

Figure 16: Number of erythrocytes containing Heinz bodies/1000 erythrocytes (Mean +/– SE) found in black skimmer (BLSK, n=108), brown pelican (BRPE, n=76), and great egret (GREG, n=91) from reference (non-shaded bars), areas of potential impact with no UV oiling (shaded bars), and areas of potential impact with trace (hashed bars), light (bars with vertical lines), and moderate (bars with horizontal line) UV oiling. Asterisks indicate significant difference ($p \leq 0.05$) from reference sites, ‡ represents significant difference ($p \leq 0.05$) from area of potential impact with no UV oiling, † represents significant difference ($p \leq 0.05$) from area of potential impact with trace UV oiling. American oystercatchers were excluded from this figure because no UV oiling data were determined from reference areas for this species. Clapper rails were excluded from this figure because of the paucity of birds from API with oil and because no Heinz bodies were identified in this species. Seaside sparrows were excluded because no Heinz body data were quantified for this species.

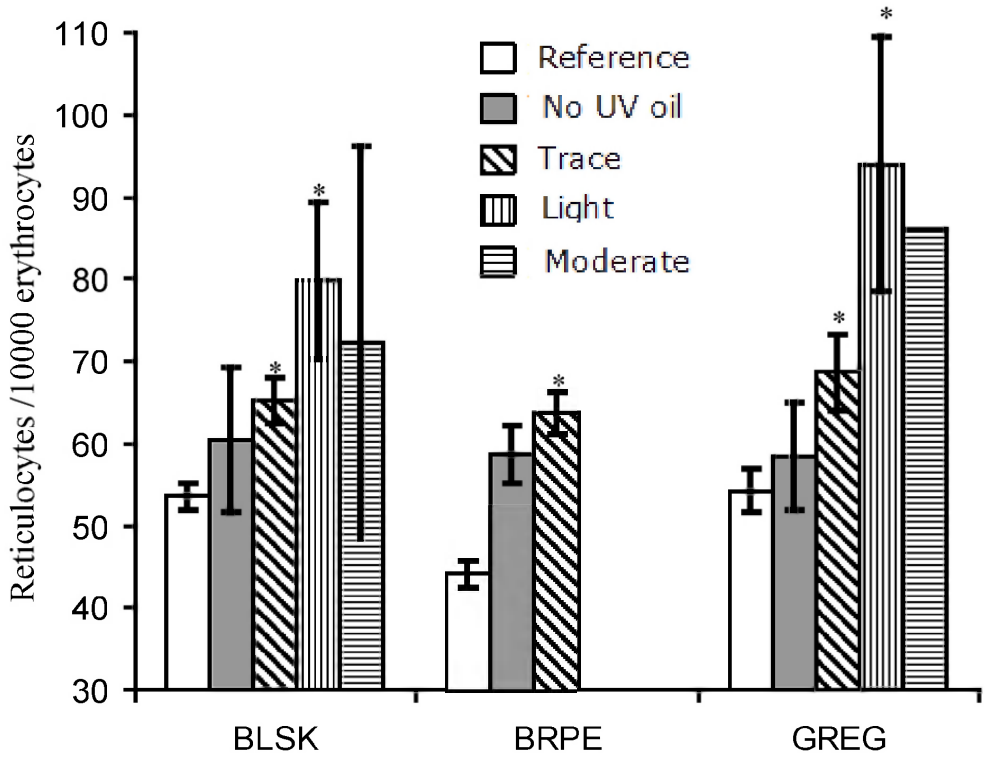

Figure 17: Number of erythrocytes containing reticulocytes/1000 erythrocytes (Mean +/– SE) found in black skimmer (BLSK, n=108), brown pelican (BRPE, n=76), and great egret (GREG, n=91) from reference (non-shaded bars), areas of potential impact with no UV oiling (shaded bars), and areas of potential impact with trace (hashed bars), light (bars with vertical lines), and moderate (bars with horizontal lines) UV oiling. Asterisks indicate significant difference ($p \leq 0.05$) from reference sites. American oystercatchers were excluded from this figure because no UV oiling data were determined from reference areas for this species. Clapper rails were excluded from this figure because of the paucity of birds from API with oil in this species. Seaside sparrows were excluded because no reticulocyte data were quantified for this species.

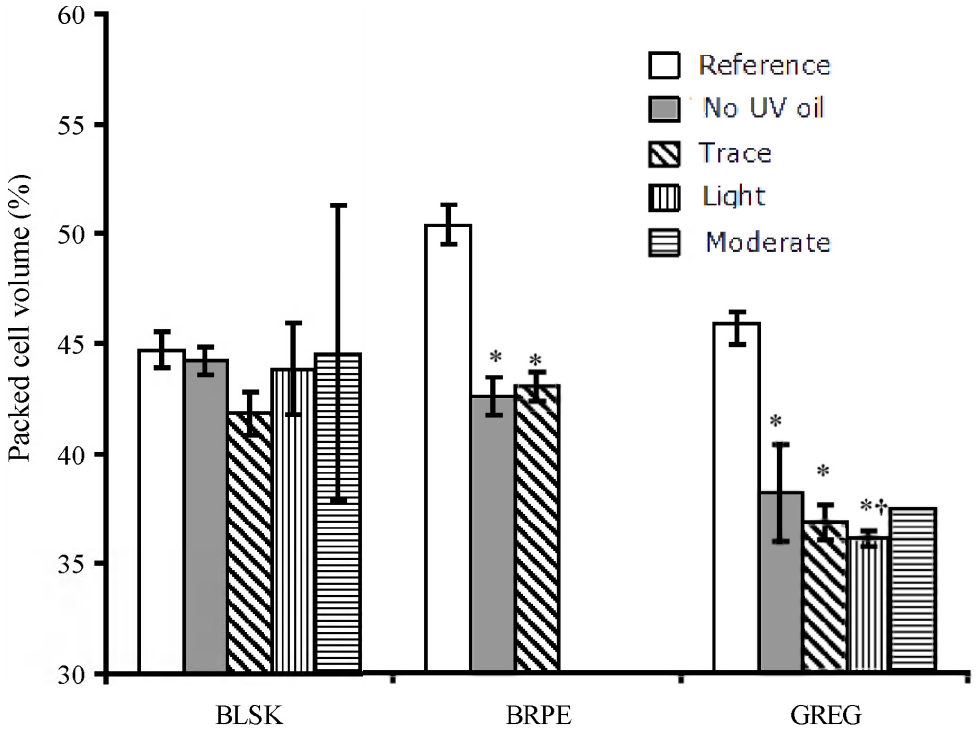

Figure 18: Packed cell volume (PCV) (Mean +/– SE) found in black skimmer (BLSK, n=103), brown pelican (BRPE, n=73), and great egret (GREG, n=96) from reference (non-shaded bars), areas of potential impact with no UV oiling (shaded bars), and areas of potential impact with trace (hashed bars), light (bars with vertical lines), and moderate (bars with horizontal lines) UV oiling. Asterisks indicate significant difference ($p \leq 0.05$) from reference sites. † represents significant difference ($p \leq 0.05$) from area of potential impact with trace UV oiling. American oystercatchers were excluded from this figure because no UV oiling data were determined from reference areas for this species. Clapper rails were excluded from this figure because of the paucity PCV data from oiled birds from API sites in this species. Seaside sparrows were excluded because no PCV data were quantified for this species.

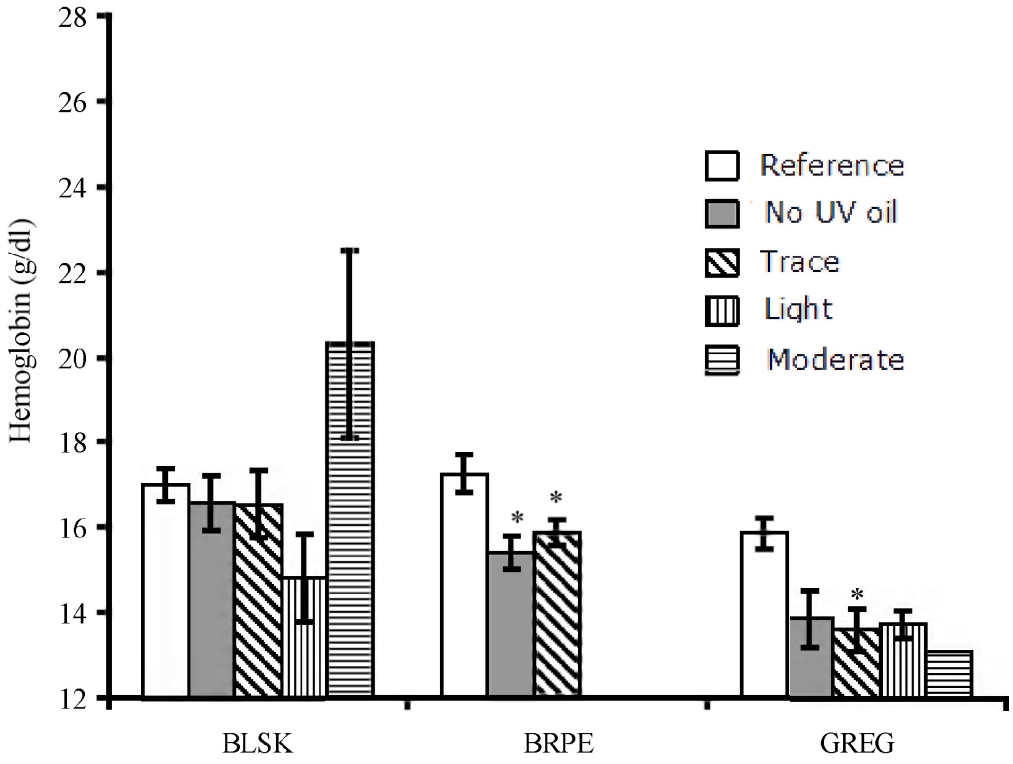

Figure 19: Hemoglobin (Hb) concentration (Mean +/– SE) found in black skimmer (BLSK, n=78), brown pelican (BRPE, n=66), and great egret (GREG, n=96) from reference (non-shaded bars), areas of potential impact with no UV oiling (shaded bars), and areas of potential impact with trace (hashed bars), light (bars with vertical lines), and moderate (bars with horizontal lines) UV oiling. Asterisks indicate significant difference ($p \leq 0.05$) from reference sites. American oystercatchers were excluded from this figure because no UV oiling data were determined from reference areas for this species. Clapper rails were excluded from this figure because of the paucity Hb data from oiled birds from API sites in this species. Seaside sparrows were excluded because no Hb data were quantified for this species.

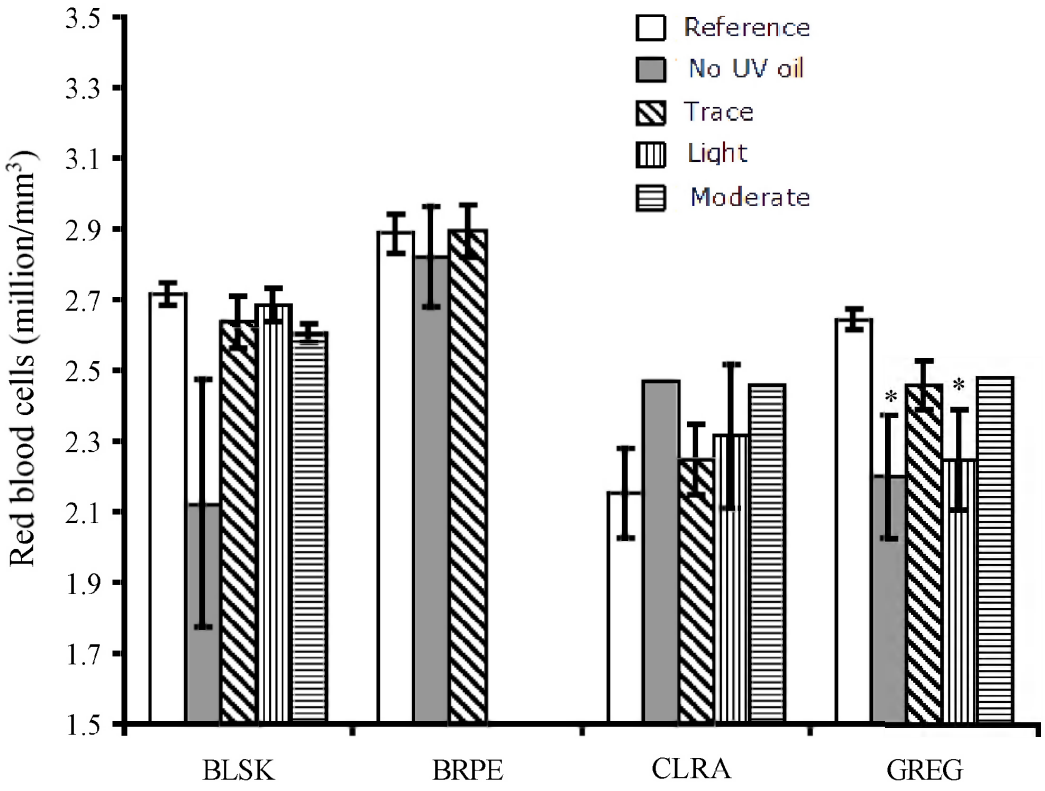

Figure 20: Red blood cell count (RBC) (Mean +/– SE) found in black skimmer (BLSK, n=93), brown pelican (BRPE, n=49), clapper rail (CLRA, n=26) and great egret (GREG, n=80) from reference (non-shaded bars), areas of potential impact with no UV oiling (shaded bars), and areas of potential impact with trace (hashed bars), light (bars with vertical lines), and moderate (bars with horizontal lines) UV oiling. Asterisks indicate significant difference ($p \leq 0.05$) from reference sites. American oystercatchers were excluded from this figure because no UV oiling data were determined from reference areas for this species. Seaside sparrows were excluded because no RBC data were quantified for this species.

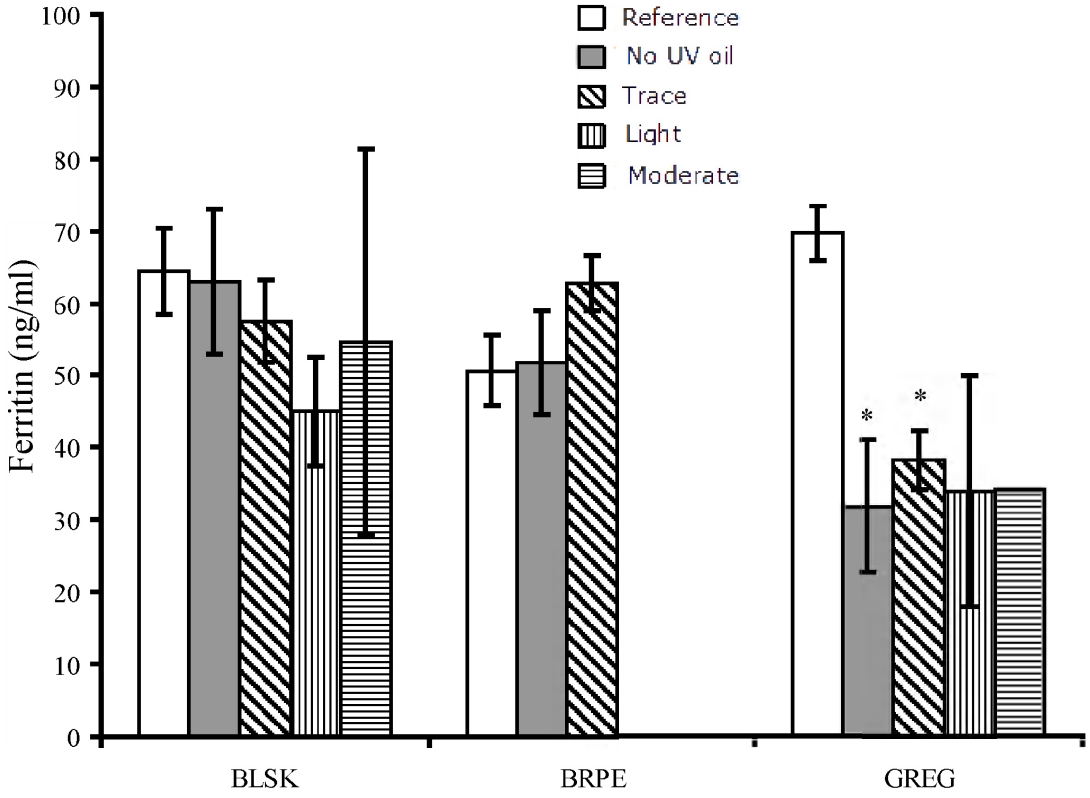

Figure 21: Ferritin concentration (Mean +/– SE) found in black skimmer (BLSK, n=69), brown pelican (BRPE, n=63), and great egret (GREG, n=85) from reference (non-shaded bars), areas of potential impact with no UV oiling (shaded bars), and areas of potential impact with trace (hashed bars), light (bars with vertical lines), and moderate (bars with horizontal lines) UV oiling. Asterisks indicate significant difference ($p \leq 0.05$) from reference sites. American oystercatchers were excluded from this figure because no UV oiling data were determined from reference areas for this species. Clapper rails were excluded from this figure because of the paucity ferritin data from oiled birds from API sites in this species. Seaside sparrows were excluded because no ferritin data were quantified for this species.

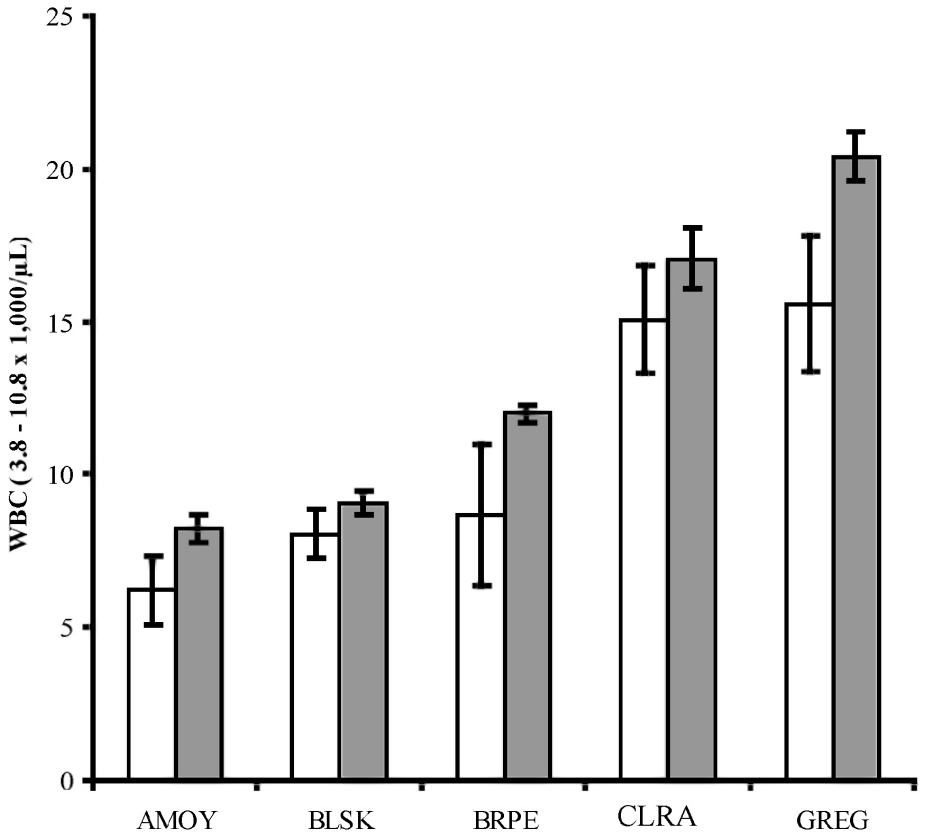

Figure 22: White blood cell count (WBC) (Mean +/– SE) for American oystercatcher (AMOY, n=33) black skimmer (BLSK, n=85), brown pelican (BRPE, n=83), clapper rail (CLRA, n=73) and great egret (GREG, n=93) without polycyclic aromatic hydrocarbons (PAH) (non-shaded bars) and with PAH (shaded bars).

APPENDIX A:

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Black skimmer

Parameter	Reference		API not visibly oiled		API oiled	
Variable	n	Mean ± SE (min-max)	n	Mean ± SE (min-max)	n	Mean ± SE (min-max)
White blood cells (×10⁹/l)	71	7.79 ± 0.40 (4-22)	33	9.03 ± 0.60 (4-18)	33	8.94 ± 0.69 (3-18)
Heterophil (%)	71	47.78 ± 1.52 (23-884)	33	39.06 ± 2.42 (12-68)	33	42.33 ± 2.46 (15-77)
Heterophils (×10⁹/l)	71	3.67 ± 0.21 (1.64-10.05)	33	3.35 ± 0.25 (1.32-7.48)	33	3.70 ± 0.38 (1.08-12.06)
Lymphocyte (%)	71	36.32 ± 1.54 (3-60)	33	48.39 ± 2.50 (12-74)	33	44.15 ± 2.45 (19-77)
Lymphocytes (×10⁹/l)	71	2.84 ± 0.19 (0.15-8.64)	33	4.58 ± 0.47 (0.6-13.32)	33	4.03 ± 0.43 (1.28-10.78)
Basophil (%)	71	3.89 ± 0.43 (0-16)	33	4.85 ± 0.65 (0-18)	33	5.18 ± 0.74 (0-19)
Basophils (×10⁹/l)	71	0.30 ± 0.04 (0-1.5)	33	0.40 ± 0.05 (0-1.10)	33	0.48 ± 0.07 (0-1.44)
Eosinophil (%)	71	11.82 ± 0.95 (0-38)	33	7.6 ± 1.22 (0-29)	33	8.21 ± 1.14 (0-22)
Eosinophils (×10⁹/l)	71	0.86 ± 0.08 (0-3.42)	33	0.67 ± 0.13 (0-3.36)	33	0.73 ± 0.12 (0-2.21)
Monocyte (%)	71	0.18 ± 0.06 (0-2)	33	0.12 ± 0.08 (0-2)	33	0.12 ± 0.08 (0-2)
Monocytes (×10⁹/l)	71	0.02 ± 0.01 (0-0.36)	33	0.01 ± 0.01 (0-0.24)	33	0.01 ± 0.01 (0-0.2)
Calcium (mg/dl)	35	8.46 ± 0.13 (7-10.1)	14	8.75 ± 0.20 (8-10)	12	10.20 ± 1.14 (8-22)
Total protein (g/dl)	35	3.90 ± 0.20 (3-47.7)	15	3.70 ± 0.34 (2.9-8)	12	3.59 ± 0.27 (2-5)
Phosphorus (mg/dl)	35	4.85 ± 0.58 (2-22)	14	4.58 ± 0.80 (1-12)	12	4.96 ± 0.93 (2-12.40)
Lactate Dehydrogenase (U/l)	37	695.08 ± 49.77 (192-3844)	14	605.64 ± 85.83 (333-1528)	12	575.92 ± 54.54 (372-873)
Creatinine Phosphokinase (U/l)	37	1542.05 ± 147.62 (192-3844)	15	2371.73 ± 721.20 (888-11936)	12	1971.50 ± 383.74 (864-5764)
Chloride (mEq/l)	34	107.26 ± 1.64 (94-126)	14	109.79 ± 2.22 (93-121)	11	105.36 ± 1.95 (89-114)
Uric Acid (mg/dl)	37	7.95 ± 1.07 (2.4-34)	15	9.60 ± 2.60 (2-36.4)	12	8.31 ± 1.60 (3-24)
Cholesterol (mg/dl)	37	256.97 ± 10.11 (116-359)	14	200.79 ± 10.41 (148-288)	12	244.75 ± 24.72 (131-396)
Aspartate Aminotransferase (U/l)	36	539.81 ± 26.45 (196-1012)	15	505.53 ± 41.71 (308-784)	12	498.25 ± 35.15 (344-776)

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Brown pelican

Parameter	Reference		API not visibly oiled		API oiled	
Variable	n	Mean ± SE (min-max)	n	Mean ± SE (min-max)	n	Mean ± SE (min-max)
White blood cells (×10^9/l)	39	10.51 ± 0.78 (5-26)	36	13.67 ± 0.87 (5-27)	21	12.62 ± 2.80 (3-67)
Heterophil (%)	39	54.36 ± 1.96 (21-78)	36	33.22 ± 1.53 (14-57)	21	30.95 ± 2.35 (14-52)
Heterophils (×10^9/l)	39	5.53 ± 0.42 (0.03-11.40)	36	4.48 ± 0.33 (1.35-9.18)	21	3.59 ± 0.58 (0.58-13.40)
Lymphocyte (%)	39	33.44 ± 1.84 (12-62)	36	50.81 ± 1.94 (22-74)	21	49.33 ± 2.69 (18-67)
Lymphocytes (×10^9/l)	39	3.61 ± 0.39 (0.63-13.52)	36	7.05 ± 0.59 (1.95-15.64)	21	5.04 ± 0.58 (0.77-12.06)
Basophil (%)	39	2.74 ± 0.33 (0-10)	36	4.06 ± 0.44 (0-9)	21	3.38 ± 0.63 (0-10)
Basophils (×10^9/l)	39	0.31 ± 0.04 (0-1.04)	36	0.53 ± 0.07 (0-1.53)	21	0.34 ± 0.08 (0-1.28)
Eosinophil (%)	39	8.08 ± 0.82 (0-18)	36	10.92 ± 1.12 (1-30)	21	15.67 ± 2.65 (5-62)
Eosinophils (×10^9/l)	39	0.84 ± 0.10 (0-2.52)	36	1.43 ± 0.17 (0.08-5.4)	21	3.24 ± 1.92 (0.21-41.54)
Monocyte (%)	39	1.38 ± 0.30 (0-6)	36	1.00 ± 0.24 (0-5)	21	0.67 ± 0.21 (0-3)
Monocytes (×10^9/l)	39	0.14 ± 0.03 (0-0.90)	36	0.17 ± 0.04 (0-1.1)	21	0.06 ± 0.02 (0-0.27)
Calcium (mg/dl)	24	9.18 ± 0.15 (7.4-10.00)	22	9.87 ± 0.12 (8.30-11)	19	9.42 ± 0.16 (9-11)
Total protein (g/dl)	24	4.40 ± 0.16 (3-6.20)	22	4.14 ± 0.13 (3-5.4)	19	3.76 ± 0.16 (3-5)
Phosphorus (mg/dl)	24	4.41 ± 0.43 (2-10.8)	22	3.59 ± 0.41 (1.7-10)	18	4.29 ± 0.47 (2-9)
Lactate Dehydrogenase (U/l)	24	613.92 ± 49.61 (300-1464)	22	735.68 ± 39.23 (456-1300)	19	747.95 ± 65.15 (411-1692)
Creatinine Phosphokinase (U/l)	24	1527.67 ± 189.80 (472-5128)	22	1514 ± 130.49 (144-3184)	19	1516.47 ± 104.67 (768-2240)
Chloride (mEq/l)	24	105.13 ± 1.46 (95-121)	22	110.73 ± 1.56 (91-123)	19	113.37 ± 1.77 (95-125)
Uric Acid (mg/dl)	24	14.78 ± 1.10 (5-25)	23	12.35 ± 0.95 (5-22)	19	11.83 ± 0.82 (4-18)
Cholesterol (mg/dl)	24	202.67 ± 9.14 (104-292)	21	149.48 ± 4.90 (104-193)*	19	153.95 ± 4.61 (116-194)*
Aspartate Aminotransferase (U/l)	24	263.12 ± 20.96 (120-508)	23	199.26 ± 13.94 (107-392)	19	214.32 ± 18.19 (111-448)

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Clapper rail

Parameter	Reference		API not visibly oiled		API oiled	
Variable	n	Mean ± SE (min-max)	n	Mean ± SE (min-max)	n	Mean ± SE (min-max)
White blood cells (×10^9/l)	78	10.95 ± 0.58 (3-28)	77	16.57 ± 0.79 (3-33)	14	13.14 ± 2.47 (2-32)
Heterophil (%)	78	35.29 ± 1.65 (9-72)	77	29.44 ± 1.52 (5-71)	14	41.21 ± 5.45 (18-72)
Heterophils (×10^9/l)	78	3.75 ± 0.25 (1.08-11.04)	77	4.45 ± 0.25 (1.20-11.40)	14	4.05 ± 0.37 (1.28-5.76)
Lymphocyte (%)	78	44.58 ± 1.55 (15-76)	77	46.88 ± 1.67 (9-79)	14	40.71 ± 5.72 (12-70)
Lymphocytes (×10^9/l)	78	4.94 ± 0.34 (0.66-14.70)	77	8.08 ± 0.52 (0.51-19.75)	14	6.76 ± 1.95 (0.56-21.44)
Basophil (%)	78	1.29 ± 0.19 (0-9)	77	1.61 ± 0.23 (0-12)	14	1.36 ± 0.39 (0-5)
Basophils (×10^9/l)	78	0.14 ± 0.02 (0-0.72)	77	0.22 ± 0.03 (0-0.54)	14	0.19 ± 0.03 (0-0.3)
Eosinophil (%)	78	18.81 ± 1.05 (0-42)	77	22.03 ± 1.21 (0-48)	14	16.64 ± 1.99 (2-29)
Eosinophils (×10^9/l)	78	2.09 ± 0.18 (0-9.52)	77	3.81 ± 0.29 (0-11.44)	14	2.21 ± 0.43 (0.02-5.2)
Monocyte (%)	78	0.03 ± 0.03 (0-2)	77	0.04 ± 0.03 (0-2)	14	0.07 ± 0.07 (0-1)
Monocytes (×10^9/l)	78	0.03 ± 0.03 (0-0.24)	77	0.003 ± 0.002 (0-0.1)	14	0.07 ± 0.07 (0-0.1)
Calcium (mg/dl)	28	11.30 ± 0.33 (9-17.4)	43	10.43 ± 0.15 (8.60-13.20)	5	11.4 ± 1.54 (8.80-17.3)
Total protein (g/dl)	29	4.47 ± 0.30 (2-11.5)	43	3.99 ± 0.17 (2.9-8.4)	5	4.44 ± 0.58 (2.7-6.2)
Phosphorus (mg/dl)	28	4.90 ± 0.38 (2-10)	43	5.32 ± 0.30 (2-11)	5	4.64 ± 1.08 (2.5-8.8)
Lactate Dehydrogenase (U/l)	28	1004.25 ± 92.40 (380-2020)	43	1411.35 ± 125.52 (519-3844)	5	804.80 ± 118.42 (508-1184)
Creatinine Phosphokinase (U/l)	29	856.931 ± 140.40 (180-3172)	43	1120.58 ± 133.46 (83-3884)	5	881.6 ± 231.17 (364-1700)
Chloride (mEq/l)	28	111.86 ± 1.64 (81-126)	43	110.38 ± 1.00 (87-122)	4	113 ± 1.47 (109-116)
Uric Acid (mg/dl)	29	10.54 ± 0.92 (5.3-24)	43	16.94 ± 1.34 (4.5-37.6)	5	13.82 ± 2.54 (5.8-18.8)
Cholesterol (mg/dl)	28	136.64 ± 4.62 (96-211)	43	137.26 ± 6.01 (88-289)	5	194.80 ± 41.61 (108-334)
Aspartate Aminotransferase (U/l)	29	899.62 ± 105.88 (284-2544)	43	932 ± 85.02 (67-2672)	5	740 ± 127.18 (496-1204)

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Great egret

Parameter	Reference		API not visibly oiled		API oiled	
Variable	n	Mean ± SE (min-max)	n	Mean ± SE (min-max)	n	Mean ± SE (min-max)
White blood cells ($\times 10^9$/l)	46	12.70 ± 0.70 (5-26)	17	15.23 ± 1.18 (6-34)	39	20.44 ± 1.41 (4-38)
Heterophil (%)	46	40.22 ± 1.84 (24-75)	17	32.65 ± 2.89 (21-61)	39	29.10 ± 1.62 (15-61)
Heterophils ($\times 10^9$/l)	46	5.04 ± 0.36 (1.50-15.00)	17	5.06 ± 0.89 (1.26-17.34)	39	5.72 ± 0.44 (1.35-12.20)
Lymphocyte (%)	46	37.63 ± 1.96 (7-67)	17	41.29 ± 2.54 (18-60)	39	43.51 ± 2.19 (15-72)
Lymphocytes ($\times 10^9$/l)	46	4.81 ± 0.38 (0.56-11.13)	17	5.86 ± 0.55 (2.64-10.35)	39	8.69 ± 0.68 (0.80-17.70)
Basophil (%)	46	3.46 ± 0.35 (0-13)	17	2.59 ± 0.60 (0-8)	39	2.97 ± 0.49 (0-17)
Basophils ($\times 10^9$/l)	46	0.43 ± 0.05 (0-1.82)	17	0.45 ± 0.13 (0-1.76)	39	0.62 ± 0.11 (0-3.57)
Eosinophil (%)	46	18.61 ± 1.25 (0-42)	17	22.94 ± 2.03 (2-33)	39	24.15 ± 1.87 (0-46)
Eosinophils ($\times 10^9$/l)	46	2.40 ± 0.22 (0-6.93)	17	3.74 ± 0.61 (0.16-9.52)	39	5.35 ± 0.63 (0-14.40)
Monocyte (%)	46	0.09 ± 0.06 (0-2)	17	0.65 ± 0.49 (0-8)	39	0.26 ± 0.15 (0-4)
Monocytes ($\times 10^9$/l)	46	0.08 ± 0.06 (0-240)	17	0.11 ± 0.11 (0-1.84)	39	0.40 ± 0.30 (0-1.08)
Calcium (mg/dl)	38	9.98 ± 0.17 (8-13)	11	10.57 ± 0.69 (8-16)	30	10.60 ± 0.23 (8-13)
Total protein (g/dl)	38	4.05 ± 0.13 (2-6)	12	4.44 ± 0.59 (2-9)	32	5.16 ± 0.27 (2.4-9.0)
Phosphorus (mg/dl)	38	5.92 ± 0.26 (3-9.20)	11	5.72 ± 0.50 (4-10)	30	5.50 ± 0.34 (2.0-9.0)
Lactate Dehydrogenase (U/l)	38	544.18 ± 51.64 (159-1640)	12	571.73 ± 55.47 (278-891)	30	804.77 ± 86.77 (147-1860)
Creatinine Phosphokinase (U/l)	38	1562.84 ± 96.07 (772-3260)	11	1760.33 ± 259.65 (756-4056)	32	1756.25 ± 167.59 (248-5752)
Chloride (mEq/l)	36	101.56 ± 1.85 (95-113)	12	109.90 ± 1.08 (103-116)*	29	112.90 ± 2.06 (71-143)*
Uric Acid (mg/dl)	38	25.52 ± 1.71 (7-50)	11	16.19 ± 2.12 (6-28)	32	16.68 ± 1.19 (5-34)
Cholesterol (mg/dl)	38	201.08 ± 4.66 (129-268)	10	126.10 ± 7.30 (85-158)*	30	124.10 ± 4.10 (93-197)*
Aspartate Aminotransferase (U/l)	38	203.63 ± 14.73 (97-526)	11	279.91 ± 80.09 (142-1068)	32	183.41 ± 10.16 (101-289)