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Reprint of: Overview of avian toxicity studies for the Deepwater Horizon Natural Resource Damage Assessment

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ABSTRACT

The Oil Pollution Act of 1990 establishes liability for injuries to natural resources because of the release or threat of release of oil. Assessment of injury to natural resources resulting from an oil spill and development and implementation of a plan for the restoration, rehabilitation, replacement or acquisition of natural resources to compensate for those injuries is accomplished through the Natural Resource Damage Assessment (NRDA) process. The NRDA process began within a week of the Deepwater Horizon oil spill, which occurred on April 20, 2010. During the spill, more than 8500 dead and impaired birds representing at least 93 avian species were collected. In addition, there were more than 3500 birds observed to be visibly oiled. While information in the literature at the time helped to identify some of the effects of oil on birds, it was not sufficient to fully characterize the nature and extent of the injuries to the thousands of live oiled birds, or to quantify those injuries in terms of effects on bird viability. As a result, the US Fish and Wildlife Service proposed various assessment activities to inform NRDA injury determination and quantification analyses associated with the Deepwater Horizon oil spill, including avian toxicity studies. The goal of these studies was to evaluate the effects of oral exposure to 1–20 ml of artificially weathered Mississippi Canyon 252 oil kg bw⁻¹ day⁻¹ from one to 28 days or one to five applications of oil to 20% of the bird's surface area. It was thought that these exposure levels would not result in immediate or short-term mortality but might result in physiological effects that ultimately could affect avian survival, reproduction and health. These studies included oral dosing studies, an external dosing study, metabolic and flight performance studies and field-based flight studies. Results of these studies indicated changes in hematologic endpoints including formation of Heinz bodies and changes in cell counts. There were also effects on multiple organ systems, cardiac function and oxidative status. External oiling affected flight patterns and time spent during flight tasks indicating that migration may be affected by short-term repeated exposure to oil. Feather damage also resulted in increased heat loss and energetic demands. The papers in this special issue indicate that the combined effects of oil toxicity and feather effects in avian species, even in the case of relatively light oiling, can significantly affect the overall health of birds.

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1. Introduction

The Deepwater Horizon (DWH) oil spill that began on April 20, 2010 initiated a Natural Resource Damage Assessment (NRDA) process under the Oil Pollution Act of 1990. As part of the DWH NRDA, a suite of avian toxicity studies was designed and implemented to inform NRDA injury determination and quantification analyses associated with the DWH oil spill. The purpose of the present paper in this special issue of *Ecotoxicology and Environmental Safety* is to present a brief description of the NRDA process under the Oil Pollution Act, general information about the DWH NRDA, a summary of DWH NRDA activities associated with the assessment of immediate effects of the spill on birds, a brief summary of the literature as it relates to the toxicity of oil in avian species, and a description of the avian toxicity studies that were conducted to inform the DWH NRDA. The other papers that comprise this special issue describe in greater detail the methodologies, specific endpoints assessed, findings and significance of the data.

2. Oil Pollution Act

The Oil Pollution Act of 1990 (OPA; Public Law 101–380 [33 U.S.C. 2701 et seq.; 104 Stat. 484]) establishes liability for injuries to natural resources because of the release or threat of release of oil into navigable waters used for navigation, adjoining shorelines, and the exclusive economic zone. The parties responsible for causing the oil spill are responsible for these injuries to natural resources. Trustees, consisting of federal, state and tribal government officials, as well as representatives of foreign governments, may act on the behalf of the public to assess injury to natural resources resulting from an oil spill and to develop and implement a plan for the restoration, rehabilitation, replacement or acquisition of natural resources to compensate for those injuries through the Natural Resource Damage Assessment (NRDA) process (Barron, 2012; Vann and Meltz, 2013a, b; Deepwater Horizon Natural Resource Damage Assessment Trustees, 2016).

3. NRDA process

There are three main phases that constitute an NRDA under the OPA: the pre-assessment phase, the restoration planning phase and restoration implementation. During the pre-assessment phase, Trustees determine if there is jurisdiction under OPA and if restoration of injured natural resources is feasible. If the Trustees determine injuries have resulted or are likely to result from the incident and response actions will not address or are not expected to address the injuries, the Trustees may prepare a Notice of Intent to Conduct Restoration Planning Activities, which is made publicly available and sent to the responsible party. The second phase in the NRDA process is the restoration planning phase, which consists of the injury assessment step and the restoration selection step. During the injury assessment step, Trustees determine the nature and extent of injuries to natural resources and the benefits they provide due to the release of oil by determining a pathway linking the incident and the natural resources (pathway) and establishing exposure to (exposure assessment) and subsequent injury of natural resources (injury evaluation) by the released oil. One approach to quantify the degree and spatial and temporal aspects of the injuries is by comparing resources and associated benefits before and after the release of oil. In the restoration selection step, information from the injury assessment is used to develop various alternatives for primary or compensatory restoration of natural resources and the benefits they provide. The Trustees then evaluate the restoration alternatives and choose a preferred alternative that typically becomes a draft restoration plan (Draft Damage Assessment and Restoration Plan), which is made available for public comment and subsequently finalized. The third phase, restoration implementation, begins after Trustees have finalized a restoration plan. Often, the final restoration plan is submitted to the responsible party for implementation or funding of the plan, which may

provide for the opportunity to settle damage claims without litigation (Hagerty and Ramseur, 2010; Barron, 2012; Vann and Meltz, 2013a, b; Deepwater Horizon Natural Resource Damage Assessment Trustees, 2016).

4. DWH oil spill NRDA

The NRDA process, as described above, began a week after the DWH oil spill. On April 20, 2010, the DWH mobile drilling unit located in the northern Gulf of Mexico (GOM) 66 km off the Louisiana coast in Mississippi Canyon 252 exploded, caught fire, and eventually sank, resulting in a massive release of oil and other substances from British Petroleum's (BP) Macondo well. The explosion and fire killed 11 workers and injured 17 others. Initial efforts to cap the well following the explosion were unsuccessful, and the uncontrolled discharge of oil and gas continued for 87 days. Approximately 3.19 million barrels (507 million liters [l]) of South Louisiana sweet crude oil were released into the ocean creating the largest offshore oil spill in the history of the United States. Cumulatively, oil on the water surface extended over an area of more than 43,300 square miles (112,100 square kilometers [km²]) and oiled more than 1300 miles (2100 km) of shoreline or more than 10% of the GOM shoreline off the coasts of Louisiana, Mississippi, Alabama and western Florida. Virtually all living resources and supporting habitats in the northern GOM were adversely affected. The overall event became known as the DWH oil spill (Corn and Copeland, 2010; Hagerty and Ramseur, 2010; Barron, 2012; Carriger et al., 2015; Deepwater Horizon Natural Resource Damage Assessment Trustees, 2016).

In response to the DWH oil spill, the US Coast Guard, on April 28, 2010, notified BP Exploration and Production and seven other entities that they were identified as potential responsible parties (Vann and Meltz, 2013a, b). The Trustee Council for the DWH NRDA is comprised of representatives from the US Department of the Interior, Department of Commerce, US Environmental Protection Agency, Department of Agriculture and the five Gulf states of Alabama, Florida, Louisiana, Mississippi and Texas. Shortly after the DWH oil spill, the pre-assessment phase was initiated by the Trustees. Impacts of the oil spill, including visibly oiled and dead birds, sea turtles and marine mammals across 2100 km of GOM shoreline had been documented by August 19, 2010. Because of this information, as well as the duration of the oil release, Trustees issued a Notice of Intent to Conduct Restoration Planning and invited potential responsible parties to participate in the NRDA with BP being the only potential responsible party agreeing to participate in the process. From 2010–2015, the Trustees conducted injury assessment activities (including data collection during the pre-assessment process) as components of the second phase of the NRDA, restoration planning. During this period, Trustees developed and implemented numerous studies to evaluate the impacts of released oil on natural resources to aid them in restoration planning. Injury assessment began immediately after the explosion and it continued as a multi-phased iterative approach such that planning and design decisions were based on data that had been collected and evaluated at the beginning of the spill (Deepwater Horizon Natural Resource Damage Assessment Trustees, 2016).

5. DWH NRDA assessment of short-term impact on birds

As mentioned above, one of the immediate impacts of the DWH oil spill was visibly oiled and dead birds. The various habitats of the northern GOM serve as important resources for over 150 species of birds. Avian species may spend their entire life cycle in the northern GOM, or they may use the area as a migratory stopover or wintering ground. Seabirds, colonial waterbirds, coastal marsh birds and shorebirds using such an area are particularly susceptible to oil spills because of their use of open water where oil tends to concentrate or because of high density along coastlines and marshes where extensive oiling can

take place. During the spill, more than 8500 dead and impaired birds representing at least 93 avian species were collected. In addition, there were more than 3500 birds observed to be visibly oiled. It is conservatively estimated that avian mortalities ranged from 51,600 to 84,500 and that lost reproduction accounted for an additional 4600 to 17,900 fledglings (Deepwater Horizon Natural Resource Damage Assessment Trustees, 2016).

As the enormity of the DWH oil spill became apparent, the US Fish and Wildlife Service (FWS) convened a team of experts to design an approach to assess potential injuries to resources for which the FWS has trust responsibilities, including migratory birds. Initial steps included the evaluation of pathways of transport and exposure, mechanisms of injury, and habitats and resources likely to be injured. Subsequent steps included the identification and design of approaches and models to estimate injury to avian resources. For birds, three primary approaches were identified to estimate mortality: Shoreline Deposition Model (SDM), Offshore Exposure Model (OEM) and Live Oiled Bird Model (LOBM). The SDM uses information on the recovery of birds on shorelines combined with information such as the deposition and persistence of birds on shorelines, the ability of survey crews to locate and recover birds, the deposition of carcasses under non-spill conditions, and other factors to estimate mortality across the affected area. The LOBM was developed to estimate injury to birds that were oiled, but did not quickly die and were not sufficiently incapacitated to enable capture by Wildlife Operations personnel. At the most basic level, the LOBM relies on three primary inputs: the numbers of birds occurring in areas affected by the oil spill (abundance); the incidence and degree to which birds are oiled (oiling rates); the fate of oiled birds (i.e., the likelihood a bird would die or suffer other adverse effects due to oil). The Trustees implemented several studies to generate these types of data for dominant bird groups, or guilds, affected by the oil spill. Field surveys were successful in documenting abundance and oiling rates for birds across the extent of the spill-affected area. However, due to a variety of reasons, definitive data required for fate estimation were difficult to obtain. Consequently, the Trustees evaluated other approaches to assess the fate of externally oiled birds. Key in this evaluation was information in the literature as well as data on oil-related health effects in field collected birds from some of the DWH NRDA pre-assessment studies (Deepwater Horizon Natural Resource Damage Assessment Trustees, 2016).

6. Toxic effects of oil on birds

While the acutely lethal effects of heavy oiling to birds are well known from oil spills worldwide (e.g., Exxon Valdez, Nestucca, Apex Houston, and various spills in the North Sea) (Burger, 1993), the physiological effects and ramifications of exposure to lesser amounts of oil are not as clear and may have greater effects on avian populations than acute mortality. When ingested by birds at less than acutely lethal dosages, oil can cause a wide range of adverse effects, including anemia, decreased nutrient absorption, altered stress response, and decreased immune function (Miller et al., 1978; Szaro et al., 1978; Holmes, 1984; Leighton et al., 1985; Leighton, 1985, 1986, 1993; Peakall et al., 1983).

6.1. Hemolytic anemia

One of the toxic effects of ingested oil in birds is hemolytic anemia (Hartung and Hunt, 1966; Eastin and Rattner, 1982; Pattee and Franson, 1982; Lee et al., 1986; Leighton et al., 1985; Leighton, 1986; Hughes et al., 1990; Yamato et al., 1996; Walton et al., 1997; Newman et al., 2000; Seiser et al., 2000; Troisi et al., 2007). Hemolytic anemia occurs by damage to hemoglobin in red blood cells associated with the formation of epoxides of polycyclic aromatic hydrocarbons (PAHs) by the action of cytochrome P450 (CYP450) monooxygenases in the liver. These reactive oxygen species cause oxidative damage to

exposed sulfhydryl groups on hemoglobin. This damage results in alterations to the tertiary structure of hemoglobin and causes precipitates to form that can coalesce into Heinz bodies. This damage is irreversible and the body can only compensate by producing more red blood cells to account for the loss of hemoglobin. If compensation is incomplete, then the bird suffers from the potentially life-threatening effects of reduced oxygen carrying capacity. Hemolytic anemia may be particularly important during migration, which is a time when metabolic oxygen demands are extremely high and hemoglobin concentrations are elevated (Piersma et al., 1996). Controlled studies have shown that hatchling and nestling herring gulls (*Larus argentatus*) can suffer from these effects within four days after oral dosing with 5 ml kg body weight (bw)⁻¹ day⁻¹ (Leighton et al., 1985; Leighton, 1986). While these studies provide some basic information on potential dosages, it represents only a small step in understanding the development and progression of hemolytic anemia in all the birds exposed to MC252 oil.

In the summer of 2010, as part of the NRDA pre-assessment, FWS initiated the Blood Physiology Study (US Department of the Interior, 2011) to determine whether hemolytic anemia was a key diagnostic feature in birds oiled during the DWH oil spill and if biomarkers of hemolytic anemia as well as other physiological and biochemical indicators of avian health were consistently related to exposure of birds to oil. In this field study, black skimmers (*Rynchops niger*), brown pelicans (*Pelecanus occidentalis*), great egrets (*Ardea alba*), and clapper rails (*Rallus crepitans*) were collected from oiled and unoiled areas and their blood was analyzed for signs of hemolytic anemia (as well as other endpoints). Approximately 35 birds of each species were collected from oiled areas, and approximately 18 to 35 birds of each species were collected from unoiled areas. The data suggested that birds with small amounts of visible oil on their feathers as well as birds collected from oiled areas with no visible oiling were experiencing hemolytic anemia based on decreased packed cell volume, increased reticulocytes and increased incidence of Heinz bodies (Fallon et al., 2014). The authors of this report concluded that while a dose-response relationship between exposure to oil and the frequency of Heinz bodies was not established, this pathology could be used for injury assessments of birds following oil spills. However, the limited scope of this pre-assessment study (hemolytic anemia and associated hematological measures) was thought insufficient to establish a definitive causal link between avian exposure to MC252 oil and injury. In addition, it was recognized that there were many other potential effects that could result from exposure to oil at doses that induced hemolytic anemia. Understanding the greater spectrum of toxicological effects at these doses was necessary to accurately characterize the injury assessment step of the restoration planning phase.

6.2. Thermoregulation

One such potential effect of exposure to oil is disruption of thermoregulation. Thermoregulation is of critical importance to homeothermic species with high energetic demands, such as migratory birds. Oil spills in colder climates have illustrated the detrimental effects of extensive oil coverage on bird survival (Perry et al., 1978). Not only does oil cause reduced buoyancy, which in turn increases the surface area of the bird exposed to cold water, but feather barbules become matted, further reducing the insulative properties of the feathers (Lambert et al., 1982; O'Hara and Morandin, 2010). In areas such as the sub-arctic, this results in rapid hypothermia and death. In more temperate regions, these changes in thermoregulatory ability have not been well studied. In areas such as the GOM, the changes in thermoregulatory ability are more likely to increase energetic demands and cause behavioral modifications (Jenssen, 1994). Birds exposed experimentally to oil for only a few hours showed increases in heat production and thermal conductance even at room temperature (McEwan and Koelink, 1973; Erasmus et al., 1981). Jackass penguins (*Spheniscus demersus*) with environmental oil exposure covering up to 70% of their

bodies had a 2.5° C drop in body temperature after only 15 min in water at 19.5–20.5° C. In water temperatures of 4.5–6° C, common eiders (*Somateria mollissima*) exposed to 70 ml of oil became hypothermic in only 70 min (Jenssen and Ekker, 1991). Sanderlings (*Calidris alba*) with 20% oil coverage of their feathers spent significantly more time preening and less time resting and were more aggressive (Burger and Tsiopoura, 1998). Environmentally exposed black-legged kittiwakes (*Rissa tridactyla*) had shorter, more frequent foraging trips following oiling (Walton et al., 1997). The consequences of increased energetic demands to maintain body temperature, combined with behavioral changes such as increased time spent preening and changes in foraging patterns, have the potential to cause weight loss, interfere with reproduction, affect the immune response and prevent optimal body condition for migration. However, there was question whether currently available information was sufficient to assess these types of injuries for live oiled birds exposed to MC252 oil that were observed during and after the DWH oil spill.

6.3. Food intake and nutrient absorption

Food intake and nutrient absorption are of critical importance in coping with changes in metabolic demand. Both increased and decreased food intake have been reported in experimentally oiled birds (Holmes et al., 1978a, b; Szaro et al., 1978, 1981; Gorsline et al., 1981; Rattner, 1981; Harvey et al., 1982; Miller et al., 1982; Pattee and Franson, 1982; Lee et al., 1985; Hughes et al., 1990; Evans and Keijl, 1993; Burger and Tsiopoura, 1998). A bird's ability to absorb nutrients and produce sufficient fat stores can be affected by a combination of factors including impairments to gastrointestinal function (Crocker et al., 1974; Hartung and Hunt, 1966; Beer, 1968; Eastin and Murray, 1981; Miller et al., 1978, 1982), inflammatory responses (Briggs et al., 1997; Newman et al., 2000) and increased metabolic rate (Butler et al., 1988). While loss of body condition is often reported in oiled birds, the extent of oiling, type of oil and length of exposure that cause these changes are not well understood. Effects on nutrient and fat utilization could be particularly important for the many birds oiled in the GOM during or prior to migration, as the ability of the birds to absorb energy from food is crucial during and immediately after the metabolically taxing process of migration.

6.4. Homeostasis

Environmental exposure to oil has also been shown to disrupt homeostatic mechanisms. The hypothalamic-pituitary-adrenal (HPA) axis plays a critical role in maintaining homeostasis. Activation of the HPA axis results in secretion of corticosterone and aldosterone from the adrenal cortex. Corticosterone is typically described as being the predominant stress hormone in birds, meaning that it coordinates physiological processes to allow the organism to respond to the stressful event. Corticosterone is responsible for re-directing resources from reproduction, reducing inflammatory responses and increasing catabolism of energy reserves. Oil ingestion has a direct effect on the adrenal cortex that results in cellular damage and affects the body's ability to mount an appropriate stress response. In birds, these effects include increased adrenal gland weight and hypertrophy (Hartung and Hunt, 1966; Miller et al., 1978, 1982; Holmes et al., 1979; Peakall et al., 1983; Leighton, 1986), changes in circulating corticosterone concentrations (Rattner and Eastin, 1981) and a blunted stress response (Gorsline and Holmes, 1982). However, it is less clear how this relates to other physiological changes following oil exposure, particularly metabolic and immune changes.

6.5. Immune and inflammatory responses

Immune and inflammatory responses are also upregulated in response to exposure to oil (Briggs et al., 1996; Perez et al., 2010).

Birds exposed to oil show increases in inflammatory responses, depressions in circulating lymphocyte numbers and immunosuppression that results in secondary infections (Fry and Lowenstine, 1985; Briggs et al., 1997; McOrist and Lenghuas, 1992; Newman et al., 2000). The irritant effects of oil on the gastrointestinal tract, combined with observed decreases in adrenal function, make it unsurprising that inflammatory responses are upregulated. Decreased circulating lymphocyte numbers could be indicative of site-specific action; however, inflammatory processes are not well understood in avian responses to oil. Further, the ability of a bird to fight off secondary infections is likely to be diminished given its immunocompromised state.

7. DWH NRDA avian toxicity studies

The information in the literature at the time helped to identify some of the effects of oil on individual systems or responses in birds. However, information from the literature and the field was not judged to be sufficient to fully characterize the nature and extent of the injuries to the thousands of live oiled birds, or to quantify those injuries in terms of effects on bird viability. As a result, the FWS proposed various assessment activities to inform NRDA injury determination and quantification analyses associated with the DWH oil spill, including avian toxicity studies that are described here. The goal of these studies was to evaluate the effects of low to moderate oil exposure and potentially repeated oil exposure that did not result in immediate or short-term mortality but might result in physiological effects that ultimately could affect avian survival, reproduction and health. Phase 1 involved development of a work plan outlining the types of studies to be conducted based on consultation with experts including avian toxicologists and pathologists, ecologists and ornithologists. During Phase 2, the studies were implemented, data were analyzed and results were reported (US Department of the Interior, 2011).

There were five groups of avian toxicity studies that were initially proposed by panels of academic, government and private sector experts convened by FWS. These studies included oral dosing studies, an external dosing study, metabolic and flight performance studies, a reproductive effects study and field-based flight studies.

The species chosen for these studies included the double-crested cormorant (*Phalacrocorax auritus*), laughing gull (*Leucophaeus atricilla*), western sandpiper (*Calidris mauri*) and rock pigeon (*Columba livia*). Double-crested cormorants were chosen because they were impacted by the DWH spill. They are common, primarily piscivorous seabirds that inhabit pelagic, coastal, and inland waterways (Glahn et al., 1995; Johnson et al., 2002) and can be used as surrogates for other piscivorous species such as pelicans (*Pelicanus* sp.), terns, and skimmers. The laughing gull is a small black-headed gull that commonly nests in large groups of up to 50,000 and was one of the most commonly oiled species found in the GOM during the DWH oil spill. Its diet consists of both terrestrial and aquatic invertebrates, and seasonal berries (Burger, 1988). The laughing gull's abundance and flexible diet makes the species a useful potential model for studying the effects of DWH oil on a broad range of species. Western sandpipers are shorebirds that have long-distance migratory routes. They were chosen because they were affected by the DWH spill and because of their applicability to methods used in metabolism and flight performance studies (Burns and Ydenberg, 2002; Nebel et al., 2013). The rock pigeon, although not a water-based bird such as those exposed to oil in the DWH spill, is a useful model to study the effects of oil exposure on flight dynamics and the metabolic challenges of migratory flight. The rock pigeon has been used to assess the effects of other toxicants on flight activity (Moye et al., 2016).

The objectives of the oral dosing studies were to develop oral dose-response relationships in multiple species that could be used to predict the toxicity of oil ingested by birds following the DWH oil spill, investigate endpoints that might be related directly or indirectly to the occurrence of hemolytic anemia to provide relevance to these

findings and to determine oral doses to be used in the metabolic and reproductive studies. The objective of the external dosing study was to develop quantitative relationships between external oiling of birds (percent cover, amount, location of oil) and internal dose. The objectives of the metabolic flight performance studies were to determine the adverse effects of external oil dosing on bird energetics and metabolism including flight performance, thermoregulation, food/energy assimilation and body composition and to measure the effects of feather oiling on flight performance. The objective of the reproductive effects study, which was not conducted, was to establish a dose-response relationship between oral doses of MC252 oil and effects on bird reproduction, including behavior, egg production, egg quality, egg viability and embryo/chick viability, health and growth. The objective of the field-based flight studies was to determine if oral and external doses of MC252 oil affected performance in homing pigeons trained for long-distance free flights between release sites and their loft.

7.1. Oral dosing studies

There were initially four oral dosing studies conducted with the double-crested cormorant, laughing gull, western sandpiper and rock pigeon. The objective of each study was to determine the appropriate dosing method for administration of artificially weathered (Forth et al., 2016) MC252 oil for that species and if dosing with 10 and/or 20 ml of artificially weathered MC252 oil $\text{kg bw}^{-1} \text{day}^{-1}$ for one or five consecutive days resulted in the development of hemolytic anemia and associated endpoints. Oil was administered by gavage in combination with a slurry of feed or immediately prior to gavage with feed. Blood was collected at periodic intervals and processed for assessment of numerous endpoints including Heinz bodies, packed cell volume, hemoglobin concentration and complete blood counts, plasma chemistries, oxidative damage and cardiac biomarkers. Birds were necropsied on day 6 with selective tissues being collected for evaluation of pathology, oxidative damage and/or CYP450 activity. Results of these initial oral scoping studies indicated that a single dose of oil had no effect within six days of dosing, but multiple doses caused significant changes in some hematology, plasma chemistry, acute phase protein and oxidative stress endpoints. However, there were no obvious signs of oil-induced anemia and it was not possible to determine which plasma endpoints could be used as indicators of oil toxicity. The appearance of oil in excreta within minutes of dosing in all species suggested that there was insufficient time for absorption of toxicants when delivered by oral gavage (Dean et al., 2017a, In this issue).

The decision was made to modify dosing methodology to more closely emulate field conditions and potentially increase absorption of oil. A second set of oral dosing studies was conducted with western sandpipers, laughing gulls and double-crested cormorants. For the sandpipers, oil continued to be administered in a feed slurry by gavage but doses were decreased from 10 or 20 ml $\text{kg bw}^{-1} \text{day}^{-1}$ to 1 or 5 ml $\text{kg bw}^{-1} \text{day}^{-1}$, and the days birds were dosed increased from four to 20. Both laughing gulls and double-crested cormorants were provided with target doses of 5 or 10 ml $\text{kg bw}^{-1} \text{day}^{-1}$ by provision of a daily allotment of oil-injected fish. Gulls were dosed for up to 28 days and cormorants were dosed for up to 21 days. The objectives of the three oral dosing studies were to determine if dosing with artificially weathered MC252 oil at target doses of 1 or 5 (western sandpipers) or 5 or 10 ml $\text{kg bw}^{-1} \text{day}^{-1}$ (gulls and cormorants) for 20–28 days resulted in the development of hemolytic anemia and associated endpoints and to identify other anatomic, hematologic, and biochemical endpoints that warranted further investigation. The same endpoints that were evaluated in the oral scoping studies mentioned above were evaluated in these oral dosing studies.

Results of the western sandpiper study were not conclusive, but the numerical decrease in hemoglobin concentration, significant increase in absolute liver weight and histological changes in the adrenal gland were consistent with exposure to oil (Bursian et al., submitted for

publication, this issue). In the laughing gull study, in addition to mortality there was a decrease in packed cell volume accompanied by evidence of Heinz bodies, increases in hepatic antioxidant endpoints and relative liver and kidney weights (Horak et al., submitted for publication, this issue). Double crested cormorants orally dosed with MC252 oil were the most severely affected of the species tested. Oil-exposed birds ate less and weighed less compared to controls. Clinical signs included reduced cloacal temperature, lethargy, feather damage, morbidity, and death (Cunningham et al., 2017, this issue). There was evidence of hemolytic anemia as indicated by decreased packed cell volume, relative reticulocytosis with an inadequate regenerative response, and presence of Heinz bodies (Harr et al., 2017a, this issue). Additionally, oil-exposed birds had oil-induced changes in complete blood count estimates and plasma chemistry and electrophoresis endpoints (Dean et al., 2017b, this issue) and increases in CYP1A protein expression and catalytic activity (Alexander et al., submitted for publication, this issue). Liver and kidney weights were increased and there was presence of lesions in kidney, liver, heart and thyroid gland (Harr et al., 2017b, this issue). Results related to changes in oxidative stress endpoints in orally dosed DCCOs are presented in Pritsos et al. (2017, this issue).

7.2. External dosing study

An external dosing study was conducted with double-crested cormorants to provide a different exposure scenario than was achieved with the oral dosing studies (Cunningham et al., 2017, this issue). External dosing allows the bird to preen oil from their feathers providing continuous exposure to experimentally simulate field exposure. The purpose of the study was to determine if oiling of 20% of the bird's surface area every three days for 21 days resulted in oil toxicity. A subset of the endpoints found to be responsive during the oral dosing study with cormorants was chosen to determine if external oiling methods produced oil toxicity. This subset of endpoints included the development of hemolytic anemia, cardiac abnormalities, and thermal stress. Results indicated hemolytic anemia was associated with Heinz bodies and reduced packed cell volume (Harr et al., 2017a, this issue), as well as increased white blood counts, monocyte and lymphocyte counts and changes in plasma chemistry endpoints (Dean et al., 2017b, this issue). Liver and kidney weights were greater in exposed birds (Harr et al., 2017b, this issue) and there was evidence of oxidative damage (Pritsos et al., submitted for publication, this issue). Evaluation of cardiac function showed decreased myocardial contractility and dysfunction and significant increases in ionized calcium in externally oiled birds (Harr et al., 2017c, this issue). Thermography indicated greater heat loss in oiled birds with an associated increase in food consumption, presumably to meet the increased energetic demand (Cunningham et al., 2017, this issue; Mathewson et al., submitted for publication).

7.3. Metabolic and flight performance studies

The metabolic and flight performance studies were conducted with western sandpipers. An external oil dosing/flight study was designed to test the effects of external oiling on takeoff and endurance flight performance. Birds were oiled externally on the wings and tail with artificially weathered MC252 oil. Takeoff performance determines the bird's ability to escape predators and was measured by inducing escape flights and measuring takeoff angle, speed and acceleration with high speed video and accelerometers. Endurance performance determines the bird's ability to make long distance flights, such as migration, and to sustain escape from predators. Flight endurance was measured in a wind tunnel under controlled conditions and energy costs of flight were measured using quantitative magnetic resonance. Results of the flight performance studies indicated that oil on the trailing edges of wings and tail reduced takeoff speed by 30% and oil on wing, tail and breast

feathers increased flight energy cost by 20–45% (Maggini et al., 2017a, this issue, Maggini et al., 2017b). A second metabolic study investigated the effects of artificially weathered MC252 oil on nighttime resting metabolic rate and thermoregulatory costs. The objectives of this study were to determine the effects of acute and three-day external oiling of 20% of the body surface on nighttime resting metabolic rate and thermal conductance, and to quantify adverse sub-lethal toxicological effects that may contribute to altered metabolic rates and thermogenic capacity under the different oil dosing regimens. The metabolic study indicated that while light feather oiling did not affect thermal conductance, there was a reduction in body temperature and induction of oxidative damage (Maggini et al., 2017c, this issue).

7.4. Field-based flight studies

Three field-based flight studies were conducted with homing pigeons to test the effects of externally applied MC252 artificially weathered crude oil on long distance flight performance. The objectives of these studies to determine the effect of external oiling on flight from 50, 80 or 100 miles over time including flight speed and flight path and whether external oiling combined with flying resulted in hemolytic anemia and associated endpoints or weight loss in the homing pigeon. A third objective was to assess any damage to feather structure on the homing pigeon due to external oiling of the feathers after multiple flights. Oil was applied to cover 25% of the surface area of the tail feathers and wings prior to release. Flight time and flight path were assessed with data loggers attached to the birds. Oiling resulted in altered flight paths, increased flight duration and increased flight distance (Perez et al., submitted for publication, this issue). Oiling also reduced the ability to regain body mass between flights (Perez et al., submitted for publication, this issue).

8. Conclusion

In the studies outlined above, exposure of selected avian species to weathered MC252 oil manifested itself in changes in hematologic parameters including formation of Heinz bodies and changes in cell counts. There were also effects on multiple organ systems, cardiac function and oxidative status. Crude oil affected flight patterns and time spent during flight tasks indicating that migration may be affected by short-term repeated exposure to oil. Feather damage also resulted in increased heat loss and energetic demands. The papers in this special issue indicate that the combined effects of oil toxicity and feather effects in avian species, even in the case of relatively light oiling, can significantly affect the overall health of birds. Findings from these studies informed the assessment of damage due to the DWH oil spill and will do so for future spills as well.

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