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Toxicological and thermoregulatory effects of feather contamination with artificially weathered MC 252 oil in western sandpipers (*Calidris mauri*)

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ABSTRACT

The external contamination of bird feathers with crude oil might have effects on feather structure and thus on thermoregulation. We tested the thermoregulatory ability of western sandpipers (*Calidris mauri*) in a respirometry chamber with oil applied either immediately prior, or three days before the experiment. The birds were then exposed to a sliding cold temperature challenge between 27 °C and −3 °C to calculate thermal conductance. After the experiment, a large blood sample was taken and the liver extracted to measure a range of parameters linked to toxicology and oxidative stress. No differences in thermal conductance were observed among groups, but birds exposed to oil for three days had reduced body temperatures and lost more body mass during that period. At necropsy, oiled birds showed a decrease in plasma albumin and sodium, and an increase in urea. This is reflective of dysfunction in the kidney at the loop of Henle. Birds, especially when exposed to the oil for three days, showed signs of oxidative stress and oxidative damage. These results show that the ingestion of externally applied oil through preening or drinking can cause toxic effects even in low doses, while we did not detect a direct effect of the external oil on thermoregulation over the temperature range tested.

1. Introduction

Oil spills affect ecosystems and wildlife in numerous ways, but perhaps the most obvious and dramatic impacts are on aquatic-associated birds. Heavy oiling of feathers makes it impossible to fly, reduces buoyancy, and greatly increases heat losses (Lambert et al., 1982; Jenssen and Ekker, 1991; O'Hara and Morandin, 2010). Internal exposure to oil by ingestion during feather preening or absorption through the skin leads to toxic pathologies (Hartung and Hunt, 1966; Eastin and Rattner, 1982; Pattee and Franson, 1982; Leighton et al., 1985; Lee et al., 1986; 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). Most heavily oiled birds die quickly. However, large numbers of birds might experience lower, non-lethal oil coverage. Such birds may experience delayed mortality or reduced fitness (Henkel et al., 2012; Montevecchi et al., 2012) because of the toxic effects of oil ingestion. However, currently available information from the literature and the field is not sufficient to characterize the nature and extent of the injuries to oiled birds, or to quantify those injuries in terms of effects on bird viability.

Studies conducted following past oil spills have focused on the physiological effects of oil ingestion; however, there are far fewer studies that focus on the physiological impacts of oil on feathers. Feathers are a unique feature of birds and through evolution they have adapted to optimize both insulation and mechanical function for flight (Parkes, 1966; Regal, 1975; Prum, 1999). In the event of contamination with oil, these functions are disrupted (O'Hara and Morandin, 2010). The overall impact and the behavioral and ecological consequences of oiling include increased preening, reductions in food consumption, weight loss and even separation from unoiled members of the flock (Larsen and Richardson, 1990; Sharp et al., 1996; Andres, 1997; Burger, 1997; Burger and Tsipoura, 1998). These studies reported low direct

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mortality, but the low quantity of oil ingested through preening is likely to have long-lasting sub-lethal effects, as demonstrated indirectly by the immediate loss of weight in oiled sanderlings (Burger and Tsipoura, 1998). To our knowledge, there are no published studies on the physiological effects of ingested and externally applied oil on shorebird thermoregulation.

Under normal conditions, birds maintain their body temperature and vital functions by producing heat metabolically. The rate of metabolic heat production is constant within a range of ambient temperatures, the thermoneutral zone (TNZ), because of regulatory changes in posture and feather insulation (Scholander et al., 1950). This constant metabolic heat production is defined as the basal metabolic rate (BMR) when the bird is in a resting, postabsorptive state within the TNZ (McNab, 1997). Below a lower critical ambient temperature, feather insulation and other passive changes are no longer enough to maintain body temperature, and additional heat has to be produced, mainly through shivering (Hohtola, 1981, 2004; Barré et al., 1985). Thus, at low ambient temperatures metabolic heat production must increase, thereby increasing metabolic rate as indicated by oxygen consumption and carbon dioxide production. Shivering metabolic rates have been shown to increase linearly as ambient temperature decreases (Scholander et al., 1950). Body size is the main factor affecting BMR in birds (Lasiewski and Dawson, 1967; Bennett and Harvey, 1987; McKechnie et al., 2006), but differences in feather insulation are responsible for differences in the slope of shivering thermogenesis, e.g. among species from different environments (Scholander et al., 1950; Hudson and Kimzey, 1966). The steeper the slope, the higher the thermal conductance of a bird will be. High thermal conductance indicates reduced insulation, increased heat loss, and thus higher energetic requirements to maintain a constant body temperature and physiological homeostasis.

External exposure to oil decreases a bird's thermoregulatory ability, which can be critical for survival, especially in cold climates (Perry et al., 1978). Oiled feather barbules become matted, resulting in a decreased insulative function of the feathers (Lambert et al., 1982; O'Hara and Morandin, 2010). For waterbirds living in cold climates, the associated effects of reduced buoyancy (which increases the surface area exposed to cold water) and reduced feather insulation can result in rapid hypothermia and death. There is not a large body of literature, however, investigating the effects of external oil coverage on waterbirds in more temperate regions, or on shorebirds in any climate. External oil exposure is reported to cause increased heat production (McEwan and Koelink, 1973; Erasmus et al., 1981) or hypothermia (Jenssen and Ekker, 1991), as well as behavioral modifications such as increased preening, increased aggressiveness, altered feeding behavior, and reduced resting time (Jenssen, 1994; Walton et al., 1997; Burger and Tsipoura, 1998). One potential effect of a reduction of feather insulation due to exposure to crude oil is an increase in thermal conductance, and the shift of the lower critical temperature (and thus the TNZ) to higher values.

External oiling also has additive toxicological effects via ingestion through preening. When ingested by birds at concentrations that are not acutely lethal, oil can cause a wide range of adverse effects, including hemolytic anemia (Leighton et al., 1985; Leighton, 1986; Yamato et al., 1996; Newman et al., 2000; Seiser et al., 2000; Troisi et al., 2007). This damage results from oxidative damage to the structure of hemoglobin causing precipitates to form that can coalesce into Heinz bodies in red blood cells (Leighton et al., 1983). Additionally, when cormorants were orally and dermally exposed to weathered MC252 crude oil external blood loss through the gastrointestinal tract due to coagulopathy was documented (Bursian et al., 2017a, in press) Animals can only compensate for this damage by producing more red blood cells to account for the loss of hemoglobin. If this compensation is incomplete, the animal suffers from a loss of the oxygen-carrying capacity of blood. This loss of oxygen-carrying capacity, along with a variety of other effects of oil on the physiological state of the bird (e.g. electrolyte imbalance, impaired organ function, oxidative damage, immune system activation) could have important negative effects on thermoregulation if oxygen limitation and other factors alter resting metabolic rate and/or reduce thermogenic capacity during shivering. Previous studies have reported increased metabolic rates in birds that ingested oil (Butler et al., 1986; Jenssen, 1994).

In this study, the patterns of heat production in western sandpipers (*Calidris mauri*) externally contaminated with oil in two scenarios were investigated: birds that had the opportunity to preen and bathe (thereby possibly drinking contaminated water), thus eliminating some oil from their feathers but ingesting it in low quantities; and birds that did not have the opportunity to preen, thus having potentially reduced feather insulation but no internal effects of oil. We hypothesized that external oil would have an effect on feather insulation resulting in an increase in thermal conductance. This should not affect BMR unless the lower critical temperature is increased so much that measurements would fall outside the TNZ. Ingestion of oil, on the other hand, may induce hypothermia or hyperthermia, thus affecting metabolic rates (including BMR) by lowering or raising the whole curve, but not affecting the slope of thermal conductance. Since these birds might still have oil on their feathers, however, a combination of both scenarios is possible in birds that were allowed to preen. We measured this using respirometry, a well-established technique that is commonly used to measure the metabolic rates of animals by accurately measuring O_2 consumption ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$), and evaporative water loss ($\dot{V}H_2O$) (Lighton, 2008). These parameters allow the determination of metabolic rate, rates of water loss, the relative contribution to energy from fat, and carbohydrate oxidation, and they allow the calculation of thermal conductance. In addition to the values directly related to thermoregulation, several endpoints that indicate toxic effects of low amounts of oil ingested when preening or drinking contaminated water were measured from blood and liver samples taken at necropsy.

2. Materials and methods

2.1. Study species

Western sandpipers are long-distance migrating shorebirds. Western sandpipers were captured near Roberts Bank and Boundary Bay in Delta, BC, Canada in July 2012 (80 individuals) under the guidelines of the University of Western Ontario Animal Use Sub-Committee (protocol 2012-027) and according to permit CA-0256 from the Canadian Wildlife Service. They were held for up to one week at Simon Fraser University (Burnaby, BC, Canada) before same-day shipment by air cargo to Toronto, ON, Canada. They were transported by vehicle to the Advanced Facility for Avian Research (AFAR) at the University of Western Ontario, London, ON Canada and maintained in captivity until experiments. These birds were initially maintained in one of the specialized 2.4 m × 3.7 m shorebird rooms at AFAR under 16 L:8D (16 h of light, 8 h of darkness) light conditions at approximately 22 °C. In 2012, during the winter, the light cycle was switched to 12 L:12D to simulate conditions on their winter range. In mid-April 2013, the light cycle was changed to 14 L:10D. The experiments were run between October 6 and October 23, 2013, corresponding to the beginning of wintering in free-ranging birds. Birds were maintained on an ad libitum diet of 80% Mazuri Waterfowl Starter and 20% trout chow (Aquamax Fingerling Starter 300). The diet was supplemented with ~50 mealworms/20 birds every other day.

2.2. External dosing

The toxicant was Mississippi Canyon 252 (MC252) oil collected during the 2010 Deepwater Horizon Gulf of Mexico oil spill and artificially weathered (Forth et al., 2017). MC252 oil was applied externally with a brush to cover 10 cm^2 of the back (2 cm^2) and belly (8 cm^2) of the experimental birds. The body cover obtained was