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Detoxification, endocrine, and immune responses of tree swallow nestlings naturally exposed to air contaminants from the Alberta oil sands

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HIGHLIGHTS

- Tree swallow boxes and passive air monitors were set up on oil sands sites.
- Air contaminants were ≥5× higher at the oil sands study sites than reference site.
- Oil sands birds had suppressed T cell response and higher EROD induction.
- Feather corticosterone levels were not different on oil sands vs. reference sites.
- Pre-fledglings compensated for physiological costs related to air contaminants.

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ABSTRACT

Changes in environmental and wildlife health from contaminants in tailings water on the Canadian oil sands have been well-studied; however, effects of air contaminants on wildlife health have not. A field study was conducted to assess biological costs of natural exposure to oil sands-related air emissions on birds. Nest boxes for tree swallows (*Tachycineta bicolor*) were erected at two sites; within 5 km of active oil sands mining and extraction, and ≥60 km south, at one reference site. Passive air monitors were deployed at the nest boxes to measure nitrogen dioxide, sulfur dioxide, ozone, volatile organic compounds, and polycyclic aromatic hydrocarbons (PAHs). Nestlings were examined at day 9 post hatching to assess T cell function and morphometry. At day 14 post hatching, a subset of nestlings was euthanized to measure detoxification enzymes, endocrine changes, and histological alterations of immune organs. Except for ozone, all air contaminants were higher at the two oil sands sites than the reference site (up to 5-fold). Adult birds had similar reproductive performance among sites ($p > 0.05$). Nestlings from industrial sites showed higher hepatic ethoxyresorufin *O*-dealkylase (EROD) induction ($p < 0.0001$) with lower relative hepatic mass ($p = 0.0001$), a smaller T cell response to the phytohemagglutinin skin test ($p = 0.007$), and smaller bursae of Fabricius ($p < 0.02$); a low sample size for one site indicating lower body condition scores ($p = 0.01$) at day 14 warrants cautious interpretation. There were no differences among nestlings for feather corticosterone ($p > 0.6$), and no histological alterations in the spleen or bursa of Fabricius ($p > 0.05$). This is the first report examining toxicological responses in wild birds exposed to air contaminants from industrial activity in the oil sands. It is also the first time that small, individual air contaminant monitors have been used to determine local contaminant levels in ambient air around nest boxes of wild birds.

1. Introduction

The oil sands comprise vast reserves of crude oil (bitumen) spanning three regions in northern Alberta and part of Saskatchewan, Canada. In 2013, 1.8 million barrels of oil per day (M bpd) were produced in the region, and production is projected to increase to 5.2 M bpd by 2030 (Canadian Association of Petroleum Producers, 2013). Bitumen remains bound to sand when mined from deposits. Water that is used to extract

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the bitumen from sand becomes contaminated (Giesy et al., 2010; Schindler, 2010), and is referred to as oil sands processed water (OSPW) which is retained in large tailings ponds (Government of Alberta, 2013).

Environmental availability of polycyclic aromatic hydrocarbons and naphthenic acids (NAs), components of OSPW, has been a major focus of recent research and monitoring in the oil sands region (Schindler, 2010). For example, studies have measured polycyclic aromatic compounds (PACs) from the Athabasca river and its tributaries (Kelly et al., 2009); have characterized and assessed the environmental fate of PAHs, NAs, and inorganic chemicals in aquatic systems (Clemente and Fedorak, 2005; Headley et al., 2001, 2005; Holowenko et al., 2002), and have measured PACs in sediments and insects (Wayland et al., 2008).

In addition, much toxicology research has focused on possible impacts of OSPW, PACs and NAs, on organisms in aquatic ecosystems in the oil sands region. For example, measurement of the induction (increases) of detoxification enzymes such as cytochrome P450 (CYP 450) through the 7-ethoxyresorufin-*O*-dealkylase (EROD) assay, has been used to determine exposure to OSPW in fish (Colavecchia et al., 2007; Tetreault et al., 2003), wood frogs *Lithobates sylvaticus* (Hersikorn and Smits, 2011), and tree swallows (Gentes et al., 2006; Smits et al., 2000). In addition, studies on immune and endocrine responses from natural and experimental exposure to OSPW and NAs have been conducted in rainbow trout *Oncorhynchus mykiss* (Leclair et al., 2013; McNeill et al., 2012), mammals (Garcia-Garcia et al., 2011), amphibians (Hersikorn and Smits, 2011), and birds (Harms et al., 2010; Smits et al., 2000).

Research regarding atmospheric emissions released from the oil sands industry, has focused on greenhouse gases (Charpentier et al., 2009; Yeh et al., 2010), on the potential acidification of lakes (Hazewinkel et al., 2008), and on the dispersion of contaminants that eventually reach aquatic systems (Kelly et al., 2010; Kelly et al., 2009). Recently, studies have reported on atmospheric air quality over the oil sands region by measuring and characterizing nitrogen oxides (NO_x), sulfur dioxide (SO_2), particulates, and several species of volatile organic compounds (VOCs) (Harner et al., 2013; Howell et al., 2013; McLinden et al., 2012; Simpson et al., 2010).

There is limited information on health effects in wildlife from contaminants from emissions relating to industrial development of the oil sands region in Canada. A meaningful approach to understand the effects of environmental contaminants is by studying sentinel wildlife species (Rabinowitz et al., 1999). Several species of wild birds are considered sentinels of environmental health and have been commonly used in ecotoxicology (Smits and Fernie, 2012). In addition, because of their highly specialized respiratory system, avian species are excellent models for studying the possible effects of airborne contaminants (Brown et al., 1997; Llacuna et al., 1993). Therefore, a field study was conducted to test the hypothesis that air concentrations of VOCs, NO_2, SO_2, O_3, and PACs, measured with passive air samplers at the nests of breeding tree swallows, would be higher at sites closer to industrial mining activity compared to reference sites, and this concentration difference would be reflected in changes in reproductive performance of adult tree swallows and physiological responses in the nestlings.

2. Materials and methods

2.1. Study sites

This study was conducted in 2012 on two industrial sites within 5 km of active mine pits, tailings ponds and processing plants on oil sands leases in Alberta, Canada. Nest boxes were erected adjacent to water bodies (5–10 m from the shorelines) to attract breeding pairs of tree swallows. The first oil sands site (OS1) is near an artificial lake constructed in 2009 from river water and does not directly receive water or materials from industrial operations. This site is approximately 65 km north of Fort McMurray, Alberta (57° 17′ 48.8″ latitude and −111° 23′ 24.3″ longitude). Nest boxes were erected at the west and east sides of this lake (approximately 500 m apart). The second oil sands site (OS2) is a pond on land reclaimed in the early 1980s, which does not receive effluent from industrial operations, and is approximately 30 km north of Fort McMurray (56° 59′ 18.1″ latitude and −111° 33′ 57.7″ longitude). The reference site pond is on public land along Highway 63, and is >60 km south of the two oil sands sites (56° 27′ 27.9″ latitude and −111° 18′ 2.3″ longitude). Totals of 38 boxes were placed at OS1, 40 at OS2, and 30 at the reference site. There was a second reference site 15 km north of the above reference site, but no birds nested there in 2012.

2.2. Reproductive performance

Nest boxes were monitored for reproductive activity (clutch initiation, clutch size, and incubation start) as previously reported (Harms et al., 2010). Boxes were left undisturbed until anticipated hatch date (12–14 days of incubation), and then monitored daily to determine hatching date (day 0), hatching success (number of eggs hatched/number of eggs laid) and brood size (number of nestlings/nest). At day 9, nestlings were identified with either a federal aluminum leg band or by coloring the feathers on one leg with a non-toxic Sharpie® marker. At day 12, fledgling success (number of fledglings/number of eggs hatched) and nest success (number of fledglings/number of eggs laid) were determined. This study was carried out under an approved University of Calgary Animal Care Committee protocol (AC12-0050). Scientific research and banding permits were obtained from Environment Canada and the Alberta Environment and Sustainable Resource Development.

2.3. Field sample collections

At days 9 and 14, nestling body mass (g) was measured with a digital scale (Tanita 1479V; Tanita Corporation of America Inc.; Arlington Heights, IL, USA), wing chord (mm) was measured with a 1-mm precision wing ruler, and size-adjusted mass was calculated (body mass/wing chord (Moore and Yong, 1991). At day 14, blood samples were collected (<1% body weight) by jugular venipuncture using 1.5 ml heparinized syringes and 25 g needles. Hematocrit and estimated total protein (TP; mg/dl; hand held refractometer; American Optical Corp., Keene, NH, USA) were obtained from the whole blood, and after centrifuging the blood samples, plasma was stored in separate aliquots in liquid nitrogen. At day 14, a subset of nestlings per box (2–3) was humanely euthanized. Nestlings were first anesthetized in a closed container with isoflurane-impregnated cotton swabs, then euthanized by cervical dislocation, and a complete necropsy was then performed. The liver, bursa of Fabricius, and spleen were excised, weighed, sampled consistently, and were either stored in cryovials in liquid nitrogen (liver) or fixed in 10% buffered formalin (spleen and bursa). Relative mass for these organs was expressed as a percentage of body mass (organ mass / [body mass − organ mass]) × 100). Sex of the birds was determined by visualization of the gonads during the necropsy. Growing feathers from the wing (primary and secondary) were plucked with forceps and stored in cryovials in liquid nitrogen for use in detoxification enzyme (EROD) assays. Secondary, tail, and body feathers were plucked manually and stored at room temperature in paper envelopes until analyzed for corticosterone.

2.4. Detoxification enzymes (EROD) in liver and feather pulp

The activity of detoxification enzymes (CYP1A) was measured by the ethoxyresorufin-*O*-dealkylase (EROD) assay based on published methods (Hersikorn and Smits, 2011; Kennedy and Jones, 1994) with modifications. All chemicals were purchased from Sigma-Aldrich Ltd (Oakville, ON, Canada) and from VWR International LLC (Edmonton,