Effects of Oil Sands Process-Affected Water on Waterfowl

by

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Abstract

The oil sands landscape in northern Alberta is interspersed with large tailings ponds that hold wastewater from bitumen mining and extraction processes. Recent monitoring results indicate that annually many thousands of birds, mostly migrating waterfowl, land on the ponds associated with this industry, but very few appear to die because of that contact. Mortalities are typically associated with bitumen exposure, which coats bird feathers to prevent flight, flotation, and thermoregulation. The recent awareness that many birds land creates an urgent need to understand the sublethal effects of contact with other pond constituents such as naphthenic acids, polycyclic aromatic hydrocarbons, and metals.

In this thesis, I reviewed the toxicological effects on birds of exposure to oil sands process-affected water and inferred potential toxicities of untested effects using a broader literature. There are few descriptions in the peer-reviewed literature of these effects, but some studies suggest that exposure to it causes reproductive disorders, alterations in endocrine and immune function, and changes in growth, metabolism, and population structure. To address the paucity of studies on waterfowl, I conducted a field experiment to emulate the repeated, short-term exposures to process-affected water that migrating water birds might experience in the oil sands. Pekin ducks (*Anas platyrhynchos domestica*) were exposed to recycled process-affected water without visible bitumen. Each exposure consisted of placing an individual bird in a plastic tub containing approximately 15 L of either process-affected water or tap water (controls) for 6–8 hours. Birds were exposed three times as juveniles and six times as adults. I assessed toxicity by evaluating body mass and a suite of biochemical, endocrinological, and hematological

analytes as well as metal residues in the birds. Results provided little evidence of toxicity. Relative to controls, juvenile birds exposed to process-affected water had higher potassium, and lower bicarbonate and cholesterol following the final exposure period, and juvenile males had a higher thyroid hormone ratio (T3/T4). Adult birds exposed to process-affected water had higher levels of vanadium and lower gamma-glutamyl transferase, and, following the final exposure period, higher bicarbonate. Adult female treated birds had higher bile acid, globulin, and molybdenum levels, whereas adult males exhibited higher levels of corticosterone. However, even for the analytes that differed significantly, means were within standard reference intervals for birds, suggesting the absence of significant biological or toxicological effects.

While it is premature to assume that ponds containing recycled water are not toxic to birds, the literature review combined with my own field experiment suggest that these ponds are substantially less dangerous than ponds containing bitumen and fresh tailings. More work will be needed to determine the generality of these results. However, for ponds that are not acutely lethal to birds and do not elicit chronic or sublethal effects, current deterrent efforts might be relaxed. This change would permit higher deterrent intensity at the more toxic ponds. This scenario contrasts with the current practices, which apply similar deterrent efforts across all types of process-affected ponds, potentially reducing, via habituation, bird protection from the constituents – bitumen and fresh tailings – that are most likely to cause mortality.

Preface

This thesis is an original work by Elizabeth Beck. The research project, of which this thesis is a part, received research ethics approval from the Animal Care and Use Committee for Biosciences in collaboration with the University of Calgary Animal Care Committee**,** "Effects Of Tailings Ponds and Reclaimed Wetlands on Waterfowl in the Oil Sands", AC11-0011, July 30, 2012.

A publication intended based on Chapter 2, and Chapter 3 of this thesis, has been submitted as E.M. Beck, J.E.G. Smits, and C.C. St. Clair. "Health of Domestic Mallards (*Anas platyrhynchos domestica*) Following Exposure to Oil Sands Process-Affected Water" to the *Journal of Environmental Science and Technology*, 2014.

Data from the first year of this research project was collected by Christine Godwin Shepard. I was responsible for 2012 data collection, analysis, and manuscript composition. Colleen Cassady St. Clair and Judit Smits were the supervisory authors, and were involved with concept formation and manuscript review.

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

OSPW	oil sands process-affected water
OSPM	oil sands process-affected material
NA	naphthenic acid
PAH	polycyclic aromatic hydrocarbon
BTEX	benzene, toluene, ethylbenzene, xylene
EDC	endocrine disrupting compound
Na	sodium
Cl	chloride
Ca	calcium
AST	aspartate aminotransferase
CK	creatine kinase
GGT	gamma-glutamyl transferase
GLDH	glutamate dehydrogenase
T3	triiodothyronine
T4	thyroxine
RBC	red blood cells
PCV	packed cell volume
WBC	white blood cell
BOD	biochemical oxygen demand
COD	chemical oxygen demand
DIC	dissolved inorganic carbon
DOC	dissolved organic carbon
TOC	total organic carbon
TDS	total dissolved solids
TSS	total suspended solids
TKN	total Kjeldahl nitrogen
perMANOVA	permutational multiple analysis of variance

Chapter 1: Introduction

1.1 Thesis Introduction

Bitumen extraction from the oil sands of northern Alberta produces large volumes of process-affected water that contains substances toxic to wildlife. Recent monitoring has shown that tens of thousands of birds land on ponds containing this water annually. This MSc study is part of a larger project, RAPP (Research on Avian Protection Project) which was created to identify ways to increase the protection of birds in the oil sands of Alberta. This project stems from a creative sentence applied to Syncrude Canada, for their failure to deter migratory birds on a tailings pond, which contained hazardous substances on April 2008, resulting in the death of 1606 ducks (Timoney and Ronconi, 2010). Large-scale mortality events are associated with birds landing in fresh tailings containing residual bitumen mats; however, a majority of ponds do not contain this type of water but still attract avian wildlife. The toxicological implications of contact with this type of water are not well understood.

1.2 Thesis Objectives

The purpose of this thesis was to investigate the impacts that oil sands process-affected water have on waterfowl. My first objective was to review the current evidence that exists to determine the risk process-affected water ponds across the oil sands region pose to birds. My second objective was to generate reliable information as to whether ducks repeatedly spending relatively short periods on oil sands process-affected water show detrimental physiological effects. This duration of contact is likely ecologically realistic to what migratory waterfowl are anticipated to experience when flying through the oil sands. To address this objective, pekin ducks were exposed to recycled process-affected water and health was assessed using a series of standard panels: biochemical, hematological, endocrinological, and metal analyses. My final objective was to determine whether exposure to process-affected water could be non-invasively detected from birds, by means of an oil adsorbent wipe as a precursor to similar identification in the feathers of wild ducks. This method could be applied to feathers collected from groups such as

hunters and aboriginal communities, which could be used to infer whether birds being shot (and potentially consumed) have been in contact with oil-sands derived products.

1.3 Thesis Outline

Following this general introduction (Chapter 1), is a review of the toxicity of process-affected water in the oil sands industry to birds (Chapter 2). The purpose of this review is to provide background for this thesis, as well as to be a self-sufficient paper to be submitted to a peer-reviewed journal. This chapter addresses my first objective. To address my second objective, in Chapter 3, I describe my primary research project. A version of this chapter has been submitted to a peer-reviewed journal. In Chapter 4, I present a general discussion.

In Appendix I, I present additional model summary information of the linear mixed models used in Chapter 3, and Appendix II consists of all data figures that were not included in Chapter 3. Appendix III consists of a description of the third objective of this thesis, although this aspect of the project was not completed.

Chapter 2: Review of the Toxicity of Process-Affected Water in the Oil Sands Industry to Birds

2.1 Introduction

2.1.1 Industry and Production of Tailings

The oil sand deposits of northeast Alberta, Canada, are home to one of the largest energy reserves throughout the globe (Timoney and Ronconi, 2010). Fifteen individual oil sands deposits which cover approximately 142,000 km^2 exist in the Athabasca, Peace River, and Cold Lake areas (Gosselin et al., 2010). The Athabasca deposit is the largest of the three, as well as the only one in which reserves are shallow enough to allow for surface mining (Gosselin et al., 2010). Both in-situ and surface mining techniques are used for the extraction of oil sands ore; techniques vary depending on the depth of the oil sand formation. Eighty percent of the existing oil sands reserves are too deep to be extracted by surface mining, but the remainder may be mined and the bitumen recovered using Clark's hot water separation process, which involves the removal of oil sand ore via a caustic hot water flotation process (Gosselin et al., 2010). The waste created during this separation process, hereafter referred to as oil sands process-affected material (OSPM) and oil sands process-affected water (OSPW) is stored in massive tailing ponds, also known as process-affected water ponds. In 2013, there were over 64 of these ponds ranging in size from less than one to over 10 km^2 and covering 176 km^2 in total (St. Clair, 2014).

Tailings ponds have many restrictions placed on them due to the toxic nature of the effluent they hold. The most of important of these is a zero-release policy stemming from *The Environmental Protection and Enhancement Act* (Alberta Government, 2010). Despite existing policies and regulations, this water can and does leak into the surrounding area (Gosselin et al., 2010). Interceptor ditches, dykes, and wells are designed to catch this seepage before it migrates into the groundwater, and return it to the tailings pond (Gosselin et al., 2010). Monitoring, such as that performed by the regional aquatics monitoring program (RAMP), has not found that process-affected water reaches the groundwater and the Athabasca River, however, more recent independent research

has detected at least 13 elements considered priority pollutants in both the river and its watershed which were attributed to oil sands activities (Kelly et al., 2010). Petroleum hydrocarbons are naturally present in the environment as both biogenic organic compounds and pyrogenic hydrocarbons which make determination of causality difficult, although recent advances in chemical fingerprinting show promising abilities to distinguish natural from anthropogenic sources (Wang et al., 2014).

Process-affected water is also contained within recycled water ponds (this water has been or will be reused in the bitumen extraction process), and is used for the formation of reclaimed wetlands. The viability of these wetlands to support biological communities of plants, invertebrates, fish, and birds has been investigated with varying conclusions. The toxicity of OSPW does decline with time and natural microbial processes, but the scale over which this occurs is lengthy, and research has found negative health consequences associated with these wetlands (Gosselin et al., 2010). For example, Hersikorn et al. (2010) showed that direct toxicity to wood frogs (*Rana sylvatica*) decreased considerably, but only after 7 years.

2.1.2 Protection Requirements and Exposure to Birds

Three sections of legislation are central to legal issues surrounding avian exposure to oil sands process-affected water. The first, under section 155 of Alberta's *Environmental Protection and Enhancement Act* (EPEA), states that hazardous substances must be stored in a manner to ensure it does not directly or indirectly come into contact with any animals (Alberta Government, 2010). This creates difficulties as the oil sands are uniquely positioned to interact with the neighboring 381,000 km^2 of boreal forest, which provides habitat for a diversity of resident bird species (Wells et al., 2008). The oil sands are also situated along converging migratory pathways used by hundreds of thousands of waterfowl each year which pass through on their way to the Peace-Athabasca Delta in Wood Buffalo National Park (Butterworth et al., 2002; Hennan and Munson, 1979). Similarly, under section 5.1 (1) of Canada's *Migratory Birds Convention Act* (MBCA), operators may not deposit substances harmful to migratory birds in any location that might be used by said birds (Government of Canada, 1994). Finally, section 32 (1) of the *Species at Risk Act* (SARA) prohibits the harm of wildlife species listed as

extirpated, endangered or threatened (Minister of Justice, 2002). In compliance with these regulations, avian deterrent strategies have been developed over the years including various types of effigies, and propane noise cannons (Timoney and Ronconi, 2010). Currently, industry relies primarily on acoustic stimuli to deter birds from landing and several new operators employ Long Range Acoustic Devices (LRADs; LRAD Corporation, San Diego, CA, USA) to convey sound up to 6.5 km into the adjacent landscape with power intensity comparable to traditional acoustic cannons (St. Clair et al., 2013). Sound pollution associated with industrial activity, specifically the LRADs, has well-known detrimental effects on songbirds (Bayne et al., 2008) which is cause for concern for the many vulnerable species that breed in the boreal forest of the region (Wells et al., 2008).

Given the scale of the oil sands and the constant accessibility of ponds on the landscape, it is effectively a near impossible task to prevent all landing events. Tailings ponds are attractive to waterfowl as they migrate because they afford opportunities to rest, refuel, and even nest. This is especially true when the ponds are ice-free in the spring due to warm water effluent, or during severe weather events, which may force birds to land (St. Clair et al., 2013). In 2011, an estimated 70,000 birds were detected during standardized monitoring sessions, and 20,540 of these detections were landings. Extrapolating a similar rate of contacts to all ponds leads to an estimate of 200,000 bird contacts with process affected water (St. Clair et al., 2013). Many species, including those listed federally as being of special concern (e.g., horned grebes), threatened (e.g., barn and bank swallows), and even endangered (e.g., whooping crane) have been observed flying through oil sands area (St. Clair et al., 2014). Mortalities have been reported for at least 43 species, primarily waterbirds such as mallards; mortalities of passerines, gulls, birds of prey, and crows have also been reported (Timoney and Ronconi, 2010). Although the ratio of mortalities to contacts is relatively low, operators are legally obliged to prevent all birds from landing.

The current legislation and approach to protection assumes that all ponds containing process-affected water have the same level of toxicity, and hence are afforded the same degree of protection for birds. However, tens of thousands of birds do land on process-affected ponds each year, and relatively little mortality is reported. Thus certain

types of OSPW might not be as harmful as once thought (St. Clair et al., 2013). For example, of the 20,540 landings, and 200,000 extrapolated contacts from 2011, only 139 mortalities were reported (St. Clair et al., 2013). The exception to this is highlighted by the recent prosecution of Syncrude under the EPEA and MBCA for failing to deter migratory waterfowl from landing on tailings ponds (Timoney and Ronconi, 2010). On April 2008, 1,606 ducks died after landing on the Syncrude Aurora tailings pond and becoming contaminated with bitumen (Timoney and Ronconi, 2010). Again, in October 2010, 547 waterfowl landed and died on Syncrude's ponds (St. Clair et al., 2012). Thus, this coarse approach to protection creates a scenario of less effective protection where it matters most.

2.1.3 Purpose of Review

Several comprehensive technical reviews have been published concerning the oil sands and their effects on species (e.g., Cruz-Martinez and Smits, 2012; Gosselin et al., 2010), however, no review on birds exists in the primary scientific literature. The purpose of this review is therefore to examine the evidence that exists to determine the toxicity of process-affected water ponds across the oil sands region to birds. Because there is very little peer-reviewed literature that addresses exactly this point, we will first draw from relevant parts of a broader literature.

2.2 Oil and Birds

Exposure to oil and oil-related products are a common occurrence in terrestrial, freshwater, and marine environments, and the toxic properties of oil have been documented in plants, invertebrates, mammals, reptiles, amphibians, and birds (Albers, 1998). Although many species are potentially affected, birds, and particularly aquatic birds, are one of the groups at highest risk (Leighton, 1993; Timoney and Ronconi, 2010).

Large-scale avian mortality events are common outcomes, particularly with crude or other heavy oil contamination, although sublethal effects with population or community level implications that extend years beyond an oiling event are also of concern (Hennan and Munson, 1979; Leighton, 1993). Examination of oil toxicity has

been conducted both in laboratory and field settings. Laboratory studies generally consist of experimental dose-responses through dietary, gavage, or mesocosm type exposures. Field based experimental studies have been conducted, although most have been in-situ sampling following marine oil spills.

Oil spills are high-risk scenarios for wildlife, especially for birds. For example, the Exxon Valdez spill (1989) killed between 100,000 to 300,000 birds (Piatt et al., 1990). Depending on their location, some oil spills have affected particular species more than others have. For example, the Apollo (1994) and Treasure (2000) oil spills off the coast of Cape Town, and the Iron Baron off the coast of Tasmania, Australia (1995) all had heavy impacts on penguin populations (The Mariner Group, 2014). Descriptions of the toxicological impacts of this type of exposure are prevalent in the literature, and a broad review was performed in 1993 (Leighton, 1993).

Birds can also be exposed to oil through contact with industrial effluent, such as oil production facility waste storage pits (Degernes, 2008). For example, at a single crude oil pit in Texas, 297 bird carcasses were recorded (Flickinger and Bunck, 1987) and, similarly, evaporation ponds in oilfield wastewater facilities in Wyoming were accountable for 269 deaths between 1998 and 2008 (Ramirez, 2010). It is worth mentioning here that in general, carcasses recovered hugely underestimate the actual number of affected birds (Ward et al., 2006). Tailings ponds are filled with waste products from industrial mining activities and those within the Alberta oil sands are the focus of this review (Allen, 2008). The oil sands are a unique example of tailings management due, in part to the scale they occupy, as well as their location.

2.3 Constituents of Concern in OSPW

A large diversity exists in the types of petroleum products and their constituent hydrocarbon species. The toxicity of these oils varies substantially and depends on a range of factors including their physical and chemical composition and characteristics, weathering conditions, the route and duration of exposure of an organism, and the bioavailability of the oil (Stone et al., 2013). Similarly, the composition of OSPW can vary substantially depending on the operator and pond type; however, some generalities can be made. Organic compounds include unrecovered bitumen, polycyclic aromatic

hydrocarbons (PAHs), benzene, toluene, ethylbenzene and xylene (BTEX), phenols, and naphthenic acids (NAs) (Allen, 2008). Inorganic fractions such as heavy metals, salts, ammonia, dissolved or suspended clay, and silt are also common (Allen, 2008).

Polycyclic aromatic hydrocarbons are a large family of naturally occurring aromatic hydrocarbon compounds found in oil deposits, but are also released during combustion of fossil fuels (Eisler, 1987). Polycyclic aromatic hydrocarbons are composed of hydrogen and carbon atoms arranged as two or more fused benzene rings (Eisler, 1987). There are thousands of PAHs, which gives the group a large range of physical and chemical characteristics; low molecular weight compounds are more soluble than heavier ones, and thus are likely those that dissolve in OSPW, and are acutely toxic to some organisms (Eisler, 1987). PAHs can also be found in soil and sediment, thus OSPM is another potential point of contact for birds. Many PAHs have exhibited teratogenic, mutagenic, and carcinogenic effects in fish, amphibians, mammals, and birds (Eisler, 1987), and have been implicated as being endocrine disrupting compounds, and as being immunotoxic (Fairbrother et al., 2004; Lintelmann et al., 2003).

Volatile organic compounds are common chemicals of concern. Specifically, the aromatics that make up BTEX are all water-soluble and are capable of bioaccumulating in the food chain, and most have documented toxic effects. For example, benzene has shown both acute and chronic toxic effects to vertebrates (e.g., hematotoxicity and leukemia) and invertebrates (CCME, 1999; Cruz-Martinez and Smits, 2012). Toluene is generally inhaled and absorbed through the lungs where it is distributed to the brain and elicits neurotoxic effects, although it can also be cardiotoxic (Cruz-Martinez and Smits, 2012). Interestingly, the toxicity of toluene is reduced when present in combination with benzene (Olsgard et al., 2008). Ethylbenzene is also poorly soluble so exposure is primarily through inhalation, although this compound has low potential for acute toxicity (Cruz-Martinez and Smits, 2012). Finally, xylenes can enter an organism through respiratory pathways, although dermal exposure is also common. Toxic effects are often linked to narcosis, although anemia, leukocytopenia, thrombocytopenia, cyanosis, and dyspnea are also documented (Cruz-Martinez and Smits, 2012).

Naphthenic acids are naturally occurring in petroleum, and concentrations in OSPW can be as high as 110 mg/L, while background concentrations in the Athabasca

region are typically less than 1 mg/L (Headley and McMartin, 2004). Naphthenic acids do not biodegrade well, so even with age they persist in OSPW, which has important considerations for OSPW in reclaimed wetlands which are built to support viable biological communities (Headley and McMartin, 2004). Naphthenic acids have been described as being one of the primary drivers of acute toxicity in fresh tailings; documented effects extend from aquatic microorganisms such as algae, to fish and mammals (Headley and McMartin, 2004). Acute high dose exposure to NAs can be lethal; other effects include cytotoxicity to both red and white blood cells, and hepatotoxicity (Cruz-Martinez and Smits, 2012). Some divergent findings show that NAs have been inaccurately represented as the most toxic contaminant. In birds, NAs have shown no effect after subchronic exposure in tree swallow nestlings (*Tachycineta bicolor*) (Gentes et al., 2007b), nor are they toxic to northern leopard frogs (*Lithobates pipiens*), in both cases at environmentally relevant concentrations (Smits et al., 2012).

Metals exist naturally within our environment, although their concentrations can be changed and redistributed with anthropogenic activities (Cruz-Martinez and Smits, 2012). Many metals are present in process-affected water (e.g., aluminum, iron, molybdenum, titanium, and vanadium) including those listed as priority and toxic pollutants (e.g., arsenic, cadmium, chromium, copper, lead, nickel, and zinc) (Allen, 2008; USEPA, 2013). It is not possible to generalize about the toxic effects of metals because they vary immensely. Some are natural constituents of an organism (e.g., essential elements), while others are only found in response to industrial activities. Some metals may elicit teratogenic effects (e.g., chromium) and others acute mortality (e.g., lead or mercury at high levels; Eisler, 2000). Mercury can also have sublethal impacts such as effects on growth, development, reproduction, and even behavior (Eisler, 2000). Further, many metals such as lead and mercury are hormetic at lower levels, meaning they have apparent positive effects (Nain and Smits, 2011).

Oil sands process-affected water is slightly salty as high concentrations of dissolved ions, especially sodium, chloride, sulfate, and bicarbonate are common (Allen, 2008). Sodium in particular can be 60 to 80 mg/L higher than would be found in natural waters (Gosselin et al., 2010). Toxic responses to the salinity of OSPW have been implicated primarily in plants and plankton communities, and this toxicity may be

additive or synergistic by the interaction between salts and naphthenic acids (Allen, 2008). High salinity has also been shown to reduce duckling growth and survival, as they are not born with functional salt glands to aid with salt processing (Swanson et al. 1984).

2.4 Toxicity of OSPW – Physical and Environmental Characteristics

It is recognized that, in addition to the actual constituents in tailings pond water, a variety of environmental and physical factors can affect its toxicity. These influences are important when considering potential effects on birds. For example, age of tailings is a well-known determinant of toxicity. There is substantial evidence that fresh tailings are acutely toxic to many aquatic organisms, from microbes to fish, and that roughly one to two years post-production the acute toxicity of tailings dissipates through microbial activities, as well as the degree of weathering (MacKinnon and Boerger, 1986). Weathering involves the evaporation and dispersion of lighter fractions leaving behind heavier fractions (Tully et al., 2009). Even with time, toxic effects of aged OSPW have still been shown in amphibians (e.g., Hersikorn and Smits, 2011; Pollet and Bendell-Young, 2000), fish (e.g., Nero et al., 2006; van den Heuvel et al., 2000, 1999), and birds (e.g., Gentes et al., 2006, 2007a; Gurney et al., 2005; Harms et al., 2010; Smits et al., 2000).

The toxicity and availability of some metals found within OSPW depend on external factors such as water salinity, pH, and hardness (Eisler, 2000). Surface tension, specific gravity, and viscosity of the oil will also affect its buoyancy and propensity to spread and flow (USEPA, 1999). Temperature is another determining factor; increased temperatures generally reduce the surface tension of a liquid, and thus oil is more likely to spread in warmer water (USEPA, 1999). In the past, containment of residual bitumen through booming minimized exposure to wildlife, however logistical challenges and the low quality of recovered floating bitumen made this approach of low economic appeal. As fresh tailings are deposited into a settling basin, they are warm, and thus the density of un-recovered bitumen will be less than water, making it condense as a floating mat. As the bitumen cools, its density will increase, such that it will sink and may escape the containment booms.

2.5 Exposure

The effects of exposure to contaminants vary depending on whether birds are resident species and subject to constant exposure, including life stages critical to growth, development, and reproduction, or if they are migratory species subject to relatively short term, but likely repeated, exposure (Cruz-Martinez and Smits, 2012). Birds that do land on OSPW may be exposed to contaminants by three major routes, external contact (dermal, percutaneous, or egg), ingestion, or inhalation.

External contamination of the surface of the bird with oil primarily leads to acute toxic effects. This contact can induce physical alteration of the interlocking feather structure, thereby compromising its waterproofing and associated air trapping and warming properties, often resulting in hypothermia (Ritchie et al., 1994). To combat hypothermia, birds must increase their metabolism, which requires an increase in energy intake. Birds have difficulty acquiring additional food resources when they are oiled, and thus may become emaciated and weak, and often die of drowning (Tully et al., 2009).

External contamination of oil to eggs from laying mothers is also a possible route of exposure (USEPA, 1999). Oil can be acutely toxic to avian embryos, other effects include decreased strength and thickness of eggshells, as well as delayed onset of hatching or overall reduced hatching success, all of which affect survival.

Exposure may also occur through ingestion of residual bitumen, either through preening (which furthers the spread of the oil on the bird) or through consumption of contaminated water. Ingestion can cause direct damage to the gastrointestinal system, which can manifest as ulcers, diarrhea, and dehydration (Tully et al., 2009). In glaucous-winged gulls and mallards, 45% of ingested petroleum hydrocarbons were excreted, while the remainder entered the blood circulation and was absorbed into the tissues (McEwan and Whitehead, 1980). Primary toxic effects usually target the red blood cells causing hemolytic anemia, but effects can extend to most body systems (Tully et al., 2009). Contaminated food is another secondary exposure route; animals in higher trophic positions may be consuming those in lower positions that have accumulated contaminants themselves (bioaccumulation of contaminants). Laboratory studies have shown accumulation and transfer of aromatics through crayfish exposed to a No. 2 fuel

oil mixture for 3 hours and fed to adult redhead ducks (*Aythya americana*) (Tarshis and Rattner, 1982). In the oil sands, birds that forage for food on the water or shoreline are at the highest risk of contacting OSPW. For example, Smits et al. (2000) found that tree swallows living on reclaimed wetlands had higher dietary (primarily invertebrate) exposure to xenobiotics relative to the reference site. Ingestion of grit (OSPM) is also a significant route of contaminant exposure, at least for mallards, particularly for compounds such as oil and grease (King and Bendell-Young, 2000).

Oil or the volatile components of oil can also be inhaled and lead to respiratory irritation and inflammation (e.g., pneumonia), emphysema, suffocation, and central nervous system effects (USEPA, 1999). There is increasing evidence that declining air quality due to airborne contaminants such as volatile organic compounds, hydrogen sulfide, sulphur dioxide, nitrogen dioxide, ozone, and particulate matter is having impacts on organisms (Cruz-Martinez and Smits, 2012), although this route of exposure is beyond the focus of this review.

2.6 Toxicity of OSPW to birds

The toxicity of OSPW to birds can be examined through a multitude of endpoints, which range from immediate mortality of individuals, through to sublethal, but chronic effects, both of which can contribute to changes in the abundance and distribution of species that can alter community structures. The acute toxicity of OSPW and its components is relatively easy to evaluate, however, the sublethal effects are more difficult to assess, as they require the ability to monitor exposed individuals for an extended, and often unknown duration. Effects of this nature might be underestimated if birds become sick, fly away, and die elsewhere. Knowledge of sublethal effects are often more interesting and relevant to the long-term survival of an individual, a population, and even a community. This is true for both resident and migratory individuals that pass through the oil sands spanning several seasons. Our descriptions of toxic effects will cover acute mortality, as well as sublethal effects involving reproductive performance, growth and survival, endocrinology, immunology, hematology and blood biochemistry, major organ systems, and community level implications (Table 2.1).

2.6.1 Acute Mortality

Some existing literature has demonstrated that the primary cause of acute avian mortality in the oil sands is direct contact with bitumen. This is likely true, as when birds contact bitumen there are acute effects similar to that of crude oil. Annual bird mortality is by no means an easy measure to compute, and many estimates exist. For example, Timoney and Ronconi (2010) estimate that 458 to 1,630 birds die each year, while Wells et al. (2008) estimates current mortalities may range from 8,000 to over 100,000 and, with a projected doubling of tailings ponds, from 17,000 to 300,000 deaths in the future. It is not our intention to comment on the accuracy of these estimates, however, it is worth noting that mass mortality events, likely those on which estimates are based, are generally associated with direct contact with bitumen and not solely with process-affected water. For example, the estimate provided by Wells et al. (2008) is founded on the assumption that all contact with tailings ponds results in oiling, and that landing events are consistent across 100 days. Thus, the probability of these mass mortality events might be overestimated because most birds are not believed to contact bitumen when they land on process-affected ponds. Others hypothesize that mortalities may be substantially underestimated as carcasses sink over time and obscure counts (Flickinger and Bunck, 1987; Timoney and Ronconi, 2010), although it seems unlikely that this effect alone would alter the reported ratio of landed to dead birds significantly.

The effects of OSPW might also be overestimated based on the substantial literature on oiled birds (conventional oils) as a proxy for oil sands interactions. Under ideal conditions, one could synthesize and apply this literature to interactions in the oil sands. However, due to a variety of methodological differences these are not relevant comparisons. One difficulty results from the variability in what is measured; even from a single blood sample, one can look at a multitude of hematological or biochemical measures. Another difference stems from the range in type and concentration of oil examined. For example, light crude oil and other light, refined products are generally more toxic than heavier crude and fuel oils, but the latter is more difficult to clean, and can spread greater distances (Stone et al., 2013). Finally, variations in exposure routes also make comparisons difficult (Leighton, 1993). The majority of previous work has

been conducted in indoor, laboratory studies and though these are important for understanding mechanisms of toxicity, field experiments are critical to understanding effects on natural bird populations.

2.6.2 Reproductive Performance

One area where the effects of OSPW might be underestimated is in the determination of reproductive toxicities. Toxicities of the reproductive systems have been described at each stage of bird reproduction, but are thought to be most damaging to eggs and juvenile birds. As previously mentioned, metals of possible concern are often in high concentrations in oil, and these can similarly affect eggs. For example, Hebert et al. (2011, 2013) examined metal and PAH trends in the eggs of waterbird nesting downstream of the oil sands. They found that the mercury burdens in the eggs of California and Ring-billed gulls have increased since earlier sampling dates (1977 and 2009 respectively). Mercury was also correlated with NA concentrations, which may suggest a common source for these substances. They suggest this might be in part because the diet of these birds primarily consists of fish, which respond quickly to fluctuating environmental mercury levels (Hebert et al., 2013). Although they did not describe any toxic effects at the levels observed, further accumulation could be harmful, as methylmercury, the main form of mercury in eggs, is known to biomagnify, thus creating the potential for detrimental effects throughout higher levels of the food chain.

Toxicants may also affect the ability of adult birds to find a mate. This can occur through behavioural alterations to courtship and mate selection, possibly through changes to sexual characteristics. For example, comparisons of the red bill spot area in yellow-legged gulls (*Larus michahellis*) exposed to the Prestige oil spill was positively correlated with body condition, and negatively correlated with levels of aspartate aminotransferase (AST), which is an enzyme released during hepatic damage, such as is seen with exposure to oil (Pérez et al., 2010). The red bill spot area is a sexually selected trait for both sexes, thus decreased coloration associated with oil exposure has potential consequences for reproductive output.

Reproductive success can also be evaluated by the abilities of adults to produce viable offspring. This can be measured by behavioral indices (e.g., food provisioning,

care of offspring), fecundity (e.g., clutch size, mass, hatching success), fitness (e.g., survival of offspring), and a variety of blood metrics which indicate normal development (e.g., hormone levels). A series of studies have examined the ecological viability of reclaimed wetlands on the oil sands using tree swallows as an indicator species. As part of this viability study, the reproductive potential, and several features indicative of parental quality and offspring health were examined. Smits et al. (2000) found no differences in reproductive success (clutch size, mass, or hatching success) between birds nesting on reclaimed wetlands and those nesting on reference sites. Again, in 2003 and 2004 similar endpoints were measured; some general trends included smaller brood sizes (2004), fewer fledged chicks (2003 and 2004), and an overall reduction in nest success (2004), but no differences in clutch size or hatching success (2003 and 2004; Gentes et al., 2006). In the same population in 2010, there were no differences between three reclaimed wetlands in terms of hatching or fledgling success, nor for the number or mass of eggs laid (Harms et al., 2010). Songbirds generally select areas to nest where they can readily forage, which is not the case around fresh tailings ponds, so this may actually help reduce possible exposure to the more threatening environments. These studies indicate the potential viability of these reclaimed wetlands to host avian species; however, some negative health effects were documented especially during poor weather conditions. If we see these effects on reclaimed wetlands, which are less toxic than ponds containing fresh oil sands effluent, then it is possible there is a serious underestimation of the effects that fresh tailings have on birds.

2.6.3 Growth and Survival

Another area of underestimation might be the effects of OSPW on growth and survival of avian species. This has been of interest in the same multi-year investigation of the viability of reclaimed wetlands previously mentioned. These have shown effects such as depression in nestling growth, no effect on survival (Gentes et al., 2007c), as well as increased mortality and decreased mass (Gentes et al., 2006). These effects are substantially compounded by harsh weather (Gentes et al., 2006) and site-related increased parasite burdens (Gentes et al., 2007c). In contrast, others have reported no effect on nestling growth in those exposed to OSPW (Smits et al., 2000) or naphthenic

acids (Gentes et al., 2007b) in the same study area. Finally, one study even reported an increase in weight and wing-lengths in birds on reclaimed sites (Harms et al., 2010), but these results occurred during seasons with ideal weather and therefore food supply.

Temporarily decreased mass and skeletal size was reported in mallard ducklings held on two oil sands wetlands compared to a reference wetland (Gurney et al., 2005). This was true following 2, 5, 9, and 13 days of exposure; however, these differences disappeared after 4½ weeks and no associated changes were detected in blood variables indicative of a physiological condition (plasma triglycerides and glycerol). Triglycerides are the primary storage form of lipids, and thus an important source of energy, and can be used to estimate the condition of a bird independent of mass (Kaneko et al., 2008). Glycerol can be indicative of starvation, as it is one of the primary products of lipolysis, and thus also an indicator of condition (Kaneko et al., 2008). The authors postulate these differences could eventually translate into decreased survival compared to reference sites and possibly impact population level recruitment. Similarly, adult Pekin ducks repeatedly exposed to recycled tailings pond water showed no difference from control birds in terms of body weight; however, measurements during the critical growing period would have been a more sensitive indicator (Beck et al., 2014).

2.6.4 Endocrinology

Endocrinology is the study of the structure and function of the endocrine organs. The fundamental role of the endocrine system is to maintain homeostasis by regulating processes involved in metabolism, growth, and reproduction (Damstra et al., 2002). This system can fall into disease status through a variety of pathways including overproduction, underproduction, transport, or clearance problems (Damstra et al., 2002; Lintelmann et al., 2003). Many tailings pond constituents, PAHs in particular, have been identified as endocrine disrupting compounds (EDCs; Lintelmann et al., 2003). Endocrine disrupting compounds are external substances capable of causing adverse health effects through changes to endocrine function (Lintelmann et al., 2003). These alterations can induce changes in many systems including behavior (e.g., parental quality), reproductive organ morphology (e.g., gonadal development, sexual ornaments), and development (e.g., eggshell quality, embryonic deformities) (Damstra et al., 2002).

Hormones, the chemical messengers of the endocrine system are secreted by endocrine glands and transported in the extracellular fluid (Lintelmann et al., 2003). Although a wide range of hormones are released, the ones often of interest are the thyroid hormones, thyroxine (T4) and triiodothyronine (T3). The ratio of T3/T4 sometimes referred to as thyroid activation (TA), can also be calculated, and is often more relevant than either of the thyroid hormones alone. Changes in thyroid function can influence traits such as feather growth, molting, behavior, and metabolism, all of which could alter and potentially compromise survival, especially in young birds (Gentes et al., 2007a). PAHs and crude oils in particular have been linked to thyroid hormone disturbances (Leighton, 1993; Smits and Fernie, 2013).

Also of interest are adrenal hormones such as the glucocorticoid corticosterone, which is commonly used as an indicator of stress. Stimuli that are perceived as stressful induce behavioral and physiological changes, collectively known as the stress response (Bortolotti et al., 2008; Palme et al., 2005). Plasma corticosterone has a very short half-life (minutes to hours), however corticosterone levels in developing feathers can provide an index of stress over an extended period of time (Bortolotti et al., 2008).

Alterations to thyroid hormones in the aforementioned tree swallow studies have been used as biomarkers of health. For example, Gentes et al. (2007a) examined the effect of OSPM on tree swallow nestlings living in nest boxes on oil sands lease sites. They found elevated plasma T3, and elevated T4 within the thyroid gland, when compared to control birds which could indicate either an increase in synthesis or retention. Another study found similar feather corticosterone levels among nestlings on reclaimed and reference sites, but when divided by sex, levels were significantly higher in male nestlings on one of the two contaminated wetlands (the youngest, most contaminated site) compared to the reference wetland (Harms et al., 2010). Similarly, though, in blood plasma, Beck et al. (2014) found that in juvenile males the thyroid hormone ratio (T3/T4) was higher in birds treated with OSPW than controls. Additionally, adult males had higher corticosterone than controls. These studies indicate that males may be under increased stress and metabolic or social demands as evidenced by higher corticosterone and relative T3.

2.6.5 Immunotoxicity

Immunotoxicity is the study of the effects of contaminants on the immune system components and function (Briggs et al., 1996). The immune system acts to resist or control the entry and spread of infectious materials, and to regulate and remove damaged cells (Ritchie et al., 1994). The nonspecific defense mechanisms (innate immunity) of birds are similar to those of mammals and include the epithelial surface, the flora of the intestinal tract, and the myeloid system (leukocytes, thrombocytes, and macrophages; Ritchie et al., 1994). The specific defense mechanism (adaptive immunity) is composed of the humoral (B cell, antibody mediated) and T lymphocyte mediated responses (Ritchie et al., 1994). The effects of chemical pollutants on an organism's immune response include those that disrupt homeostatic mechanisms such as endocrine pathways, or reduce the ability to fight pathogens. Because the immune system is not localized, toxicants can secondarily affect such things as reproduction, development, and growth (Briggs et al., 1996). Tests of immune function allow assessment of subclinical changes to avian physiology and health.

Three studies have investigated the immunotoxic effects of OSPW on tree swallows. Smits et al., (2000) examined the T lymphocyte proliferative response (PHA test; cell mediated immune response (CMI) across 2 years. In 1997, the CMI response of nestlings in one of the experimental wetlands was higher than on another, but not from the reference site, whereas in 1998, there were no differences among any of the sites. Methods in the second year were more refined (dosage and measurement tools) and thus possibly more accurate, or the T cell proliferative response was not sensitive enough to the compounds to which these birds were exposed (e.g., PAHs, NAs). The authors conclude that consistent trends pointed towards reference birds exhibiting a stronger immune response; however, further testing of other aspects of immune function was necessary. Harms et al. (2010) found that tree swallow nestlings in reclaimed oil sands wetlands had a greater delayed-type hypersensitivity (DTH; an integrated evaluation of cell-mediated immune function) response than those nesting on a natural wetland. They did not find any differences in the innate immune response (chemiluminescence assay). Gentes et al., (2007c) used mean parasite infestation (blow flies, *Protocalliphora* spp.) as

a proxy for immune function. They found that nests on reclaimed wetlands had 60–72% more parasites and nestlings on these sites had double the parasite load when compared to the reference site. Indirect tests of immunotoxicity such as these can be more biologically relevant (Smits and Fernie, 2013).

2.6.6 Hematology and Biochemistry

A critical component of innate immunity involves the myeloid system from which the cells responsible for innate immunity originate. Hematology is the study of the blood cells, with the most common test in hematology being the CBC (complete blood cell count) which provides information as to the number, size, characteristics, and volume of white blood cells (WBC), red blood cells (RBC), and platelets (Tully et al., 2009). Hematology has been used to reflect oil induced stresses such as hemolytic anemia (Leighton, 1993). Information on damage to or the functional capacity of organ systems can be examined through plasma or serum chemistry. Specific and sensitive indicators of disease vary by species, but usually include those that inform on the liver, (e.g., gamma-glutamyl transferase - GGT, glutamate dehydrogenase - GLDH, AST, cholesterol, and bile acids), kidney (e.g., blood urea nitrogen, uric acid, potassium, sodium, and phosphorus), and muscle damage (creatine kinase - CK). Elevations in enzymes have been reported, but not consistently in the crude oil literature (Leighton, 1993).

One study examined several blood biochemical variables (proteins, cholesterol, GLDH, CK, AST, bile acids, glucose, and uric acid), but found only higher total proteins in birds treated with NA when compared with controls, and in this treatment group, proteins were positively associated with hematocrit which may have been more related to hydration status than toxicant exposure (Gentes et al., 2007b). Another study found no differences in plasma triglycerides, but higher glycerol in 13-day-old ducklings compared to a reference site (Gurney et al., 2005). In another waterfowl study, Beck et al. (2014) found that juvenile Pekin ducks exposed to recycled OSPW had higher potassium following the final of a series of exposures, and the same was found for bicarbonate in adult ducks. Also in adults, female treatment birds had higher globulins, bile acids, and all adults had lower GGT than controls. However, in all cases of statistical differences, means were not outside normal reference ranges, so they could not be considered

biologically or toxicologically important differences. In this study, metal and mineral concentrations were also tested, and vanadium was found to be significantly higher in birds exposed to OSPW than in controls. Little is known about the toxicity of vanadium to birds, although one chronic dietary study with mallards revealed some changes to blood chemistry, as well as evidence of intestinal hemorrhage and hepatic oxidative stress, although confounding factors such as weight loss may have been partially responsible for the changes observed (Rattner et al., 2006).

2.6.7 Examination of Major Organs

Damage to the functional capacity of organ systems can also be examined through ethoxyresorufin-o-deethylase (EROD), relative organ weights, and histology. EROD is a measure of detoxification effort secondary to exposure to contaminants, however contaminants can be absorbed, but not result in increased EROD. In the oil sands, tree swallow nestlings had higher EROD activity, (Gentes et al., 2006; Smits et al., 2000). Conversely, those dosed with naphthenic acids did not show any changes to EROD, nor were the weights of major organs (liver, heart, spleen, and bursa) different among experimental groups (Gentes et al., 2007b). This same study also examined the histology of the major organs, but found no indications of adverse effects.

2.6.8 Population and Community Effects

Population parameters in oil-affected species are often poorly understood due to the scale over which they occur. Local population sizes are generally reduced immediately following large marine oiling events primarily because of acute mortality. For example, common scoter population fell from 17,650 to 4,768 the winter following the Sea Empress spill (Banks et al., 2008). Although some species may be able to rebound after an oil spill, such as the above case in which populations were up to pre spill numbers 10 years after the event, others have reported less successful recoveries. For example, Iverson and Esler (2010) used demographic and survey data to estimate population level implications of the Exxon Valdez oil spill on harlequin ducks. They estimate a recovery period of 24 years for the population to recover, emphasizing that effects of chronic exposure such as reduced demographic performance can have larger

impacts on populations than acute mortality. Exposure to oiled water can also decrease avian reproductive rates. For example, mortality of adults can result in disruption of pair bonds leading to decreased breeding success, and mortality of eggs and chicks can lower population recruitment (Crawford et al., 2000).

In the oil sands, Dagenais (2008) found an overall reduction in species richness between 1976–1983 and 2006–2007 bird surveys, as well as different community composition. This was especially true for substrate nester guilds (cavity, tree, and water). Although there exists a good body of information on bird populations in the neighboring boreal forest, there are no other studies on this in the oil sands that are specific to OSPW.

2.7 Research Recommendations

In addition to the need for future research, the development of a useable framework for the assessment of avian effects, such as one including initial exposure assessment, followed by risk assessment and management would be most beneficial (Hart et al., 2001).

Some areas of investigation might include the potential for genotoxic, carcinogenic, and neurotoxic effects. Many of these have been documented for PAHs, but not tested for OSPW. However, carcinogenesis is not a great concern in wildlife that generally live a few years only, that is largely a human concern. Neurotoxicity has been examined through the surrogate of behavioural studies, and the fact that animals with neurological problems will show abnormal courtship behaviour, feeding, tending of young, predator avoidance, or migratory behavior. Another area of interest may be how contaminants affect sexual selection. Impairment of sexual signals or sexual ornamentation can also have population level consequences, which are important to our understanding of species persistence in the oil sands. Changes to species richness are also very important from an ecosystem health perspective, but except for Dagenais (2008), have not been explicitly tested. Long-term studies involving the potentially latent or long-term effects of exposure, such as seen with studies of mercury trends in eggs, and tree swallows systems, are also yet to be understood. Cruz-Martinez and Smits (2012) recommend at least two-year duration for field studies to account for natural fluctuations and variability.

The majority of previous work has examined reclaimed wetlands, while one other has looked at the acute toxicity of naphthenic acids, and one recycled water pond. Examination of a wider range of ponds would be beneficial for making management decisions related to bird protection. Although we focused on aquatic toxicity, the effects of airborne contaminants can also be a significant route of exposure, and are conspicuously lacking in the oil sands (Cruz-Martinez and Smits, 2012).

2.8 Conclusions

Tailings ponds are a fixture in the oil sands, and they will exist as long we have need of bitumen energy reserves. The mass waterfowl mortalities in 2008 and 2010 highlighted the need for better deterrence of birds from oil sands process-affected water, but also a better understanding of the toxicity of this water. Due to the enormity of the scale these ponds occupy, it is not possible to prevent all birds from landing. Therefore, there needs to be prioritization in preventative measures based on a risk vs. effort analysis to maximize their effectiveness.

Aside from direct mortality, potential toxic effects, or early warning signs of such effects documented in the oil sands, include EROD activation, reduced reproductive performance, disrupted thyroid hormones, and evidence of immunotoxicity in tree swallows. Also evident were growth alterations in waterfowl raised on reclaimed wetlands, increased egg mercury concentrations in water birds nesting on lakes downstream of the oil sands, increased vanadium concentrations in mallard blood exposed to recycled OSPW, and an overall reduction in bird species richness on oil sands wetlands when compared to earlier survey years.

Constituents of concern in OSPW include residual bitumen, PAHs and other hydrocarbons (e.g., BTEX), NAs, metals, and salts. It is well recognized that the result of landing in bitumen or fresh tailings where constituents of concern are in their highest concentrations will be acute mortality, thus ponds, or sections of ponds containing these substances can be considered to pose the highest risk to birds. However, aged OSPW contained within other pond types, such as recycle water ponds, generally do not contain such high concentrations of these compounds. Although the toxicity of these other ponds is not well understood, it is by no means comparable to those containing fresh tailings

birds are afforded the same degree of protection, resulting in a unequal allocation of effort based on risk.

The ecological issues and social concerns central to the oil sands are not unique to Alberta, or even Canada. In terms of societal sensitivity, mallards and other waterfowl are favored by human hunters as well as non-hunters in this and other areas. Thus, whether people are eating these game birds or simply appreciating them for their ecological or environmental value, they are important. Further understanding is also important for human and environmental health as well. There is a longstanding history of using animals as ecological indicators, or monitors, and there is potential for this in the oil sands region.

In both past and present times, research and management efforts have been led by federal and provincial government regulatory bodies, government, and arm's length monitoring programs, environmental consulting firms hired by oil sands operators, as well as independent research through academia. The integration of people, knowledge, and resources, and the standardization of research and data collection are critical to future efforts of maintaining economic prosperity while decreasing the ecological footprint of the oil sands industry.

Table 2.1: Summary of effects on birds in the oil sands			
Reference	**Species and Exposure**	**Description**	**Significant Findings**
Gurney et al. (2005)	Juvenile mallards raised on oil sands wetlands	• Body morphology (skeletal size and mass) • Body composition (liver and spleen mass) • Physiological condition (plasma metabolites) • Toxicant exposure (PAH and EROD) • Hematology	• Smaller, with lower body mass • No effect on triglycerides • No effect on EROD • Higher PAHs • No effect on hematology
Smits et al. (2000)	Tree swallows living on oil sands wetlands	• Reproductive performance (success and growth) • Immune function • EROD • Nesting growth and survival	• No effect on reproduction, immune response, growth, or survival • EROD induction
Gentes et al. (2006)		• Reproductive performance (clutch size, hatching success, fledglings/nest, fledging success, nest success) • Nestling growth (weight and wing length), survival • EROD	• No difference in clutch size or hatching success • Reduced fledglings /nest, fledging and nest success, greater mortality • Smaller brood size, reduced weight, elevated EROD
Gentes et al. (2007a)		Endocrinology (T3, T4)	• Elevated plasma T3 and T4 in the thyroid gland
Gentes et al. (2007c)		• Immune function (blow fly) • Nesting growth and survival	• 60–72% more larvae • 2 x burden in nestlings • Decreased mass • No effect on survival
Gentes et al. (2007b)	Tree swallows living on oil sands wetlands exposed to NAs	• Growth • Hematology and biochemistry • Histology and organ weights • EROD	• No effect on growth, PCV, biochemistry, organ weights, or EROD
Dagenais (2008)	Breeding birds living on oil sands wetlands	Species richness and community composition	Reduced species richness and community composition from historical records

Harms et al. (2010)	Tree swallows living on oil sands wetlands	• Reproductive performance (hatching and fledgling success, egg number and mass) • Body condition (growth, weight, skeletal measurements) • Immune function • Stress	• No effect on reproduction • Stronger immune response in tree swallows on older oil sand wetlands • Higher feather corticosterone in males from the most contaminated site
Hebert et al. (2011)	Ring-billed and California gulls, Caspian and Common terns nesting downstream of the oil sands	Mercury levels in eggs	• Low PAHs and arsenic • All had measurable mercury • 40% increase over 22 years • Higher in elevated trophic positions • Hg correlated with naphthalene
Hebert et al. (2013)			• Gulls, increased in 2012 from earliest sampling year • Terns, no significant increase • Gulls and common tern below toxic threshold, Caspian tern above
Beck et al. (2014)	Pekin ducks exposed to recycled OSPW	• Body mass • Biochemistry and endocrinology • Hematology • Metals	• No evidence of toxicity on body mass, biochemistry, endocrinology, or hematology • Higher vanadium

Note: Hatching success (hatched/eggs laid); Fledglings/nest, fledging success (fledged/ hatched), nest success (fledged/laid). Tree swallows (*Tachycineta bicolor*), mallard (*Anas platyrhynchos*), pekin duck (*Anas platyrhynchos domestica*), ring-billed gull *(Larus delawarensis),* California gull *(Larus californicus),* Caspian tern *(Hydroprogne caspia),* and common tern *(Sterna hirundo).*

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Chapter 3: Health of Domestic Mallards (*Anas platyrhynchos domestica*) Following Exposure to Oil Sands Process-Affected Water

3.1 Introduction

The Alberta oil sands underlie an area of 140,000 km^2 with bitumen deposits that comprise one of the largest crude oil reserves in the world (Gosselin et al., 2010). Bitumen is a mixture of organic compounds and trace metals that can be upgraded into more valuable forms of fuel such as crude oil (Gosselin et al., 2010). Approximately 20% of this resource can be extracted via surface mining; bitumen is recovered from oil-impregnated sand using Clark's hot water separation process which requires large amounts of water (the typical ratio is 3:1; Allen, 2008). Some of this water is reused in the mining process, but it has accumulated over the past four decades and includes by-products that cannot be discharged from mine sites (Alberta Government, 2010). Sixty four oil sands process-affected water (hereafter OSPW) ponds ranging in size from less than 0.1 to 929.6 ha with a total surface area of 182 km^2 currently exist (Alberta Government, 2013; St. Clair, 2014). These ponds may contain residual bitumen, fine clay particulate, and several other mining by-products including polycyclic aromatic hydrocarbons, naphthenic acids, salts, ammonia, and trace metals (Allen, 2008).

Although the specific constituents of process-affected water ponds vary with age and operator-specific mining procedures, all are considered harmful to wildlife, including invertebrates (e.g., Wiseman et al., 2013), amphibians (e.g., Pollet and Bendell-Young, 2000), fish (e.g., McNeill et al., 2012), mammals (e.g., Rogers et al., 2002), and birds (e.g., Hennan and Munson, 1979). Over one million migratory water birds pass through the oil sands region each spring and fall travelling to and from the Peace-Athabasca Delta 200 km north (Hennan and Munson, 1979). The Delta is a globally significant staging area that hosts waterfowl from across North America (Butterworth et al., 2002). The oil sands industry is obliged by federal and provincial laws to mitigate the risks that process-affected water ponds pose to migratory birds. Deterrent systems have been composed of visual stimuli such as floating effigies and/or auditory stimuli such as propane cannons (Ronconi and St. Clair, 2006). The issue of bird protection attracted minimal public attention until mass mortalities of migrating flocks occurred in 2008 and 2010 (R. v.

Syncrude Canada Ltd., 2010), which prompted provincial regulators to implement a standardized regional bird monitoring program to monitor bird contacts with process-affected water ponds (Ronconi, 2011). In 2012, over 20,000 such contacts were reported.

The scale on which birds appear to use process-affected water ponds is inconsistent with the legal requirements imposed on the industry to prevent bird contact altogether (Alberta Government, 2010; Government of Canada, 1994). Thus, there is an urgent need to understand the biological effects of pond contaminants on the birds that land. Toxic effects of petroleum products on birds are well documented but primarily address compounds associated with conventional oil and accidental oil spills rather than the constant accessibility to waste products from process-affected water ponds in the oil sands (Leighton, 1993). Disasters such as the Exxon Valdez spill in Prince William Sound (1989), the Prestige spill off the coast of Spain (2002) and the Deepwater Horizon spill in the Gulf of Mexico (2010) have produced most of the literature concerning oil-bird interactions. Mortality is likely for birds that come into contact with crude oil or residual bitumen (as is the case in the oil sands), because both adhere to feathers, destroying their waterproofing and insulating properties and hindering thermoregulation, buoyancy, and flight (Leighton, 1993; Ritchie et al., 1994). Additional toxic effects stem from ingestion of oil, bitumen and other chemical compounds during preening or through contaminated food or water (Leighton, 1993). Ingestion of small amounts of bitumen can induce a range of toxicities affecting gastrointestinal, hematological, immunological, genetic, neurological, reproductive, and developmental systems (Leighton, 1993). More subtle effects include hormonal disruptions and behavioral changes.

Unlike the substantial body of work on crude oil, the results of the exposure of birds to process-affected water that does not contain fresh tailings or residual bitumen, which is the case for recycled OSPW, are rarely addressed, although this type of water also occurs in reclaimed wetlands (Bendell-Young et al., 2000). A single experiment with mallard ducklings reared on wetlands containing oil sands effluent suggested that such water is not acutely toxic (Gurney et al., 2005). This possibility is supported by the relative rarity of mortalities – less than 1% of the number of contacts – reported in 2012 (St. Clair et al., 2013).

The objective of this study was to determine whether pekin ducks (*Anas platyrhynchos domestica*), a sub-species of mallards, exhibit adverse physiological health effects after repeated, but brief, contact with recycled OSPW.

3.2 Materials and Methods

3.2.1 Bird Acquisition and Housing

We used pekin ducks for our exposure experiments because they are closely related to wild mallards, which are abundant in the oil sands region.(Hennan and Munson, 1979) Ducks in general are subject to intense scrutiny, as they are most likely to land in large numbers on process-affected water ponds during migration stopovers. We obtained 36 ducklings from a commercial hatchery (Golden Feather Hatchery, Chilliwack, BC, Canada) in July 2011. The ducklings were held in a field camp (Lewyk Campground, Fort McMurray, AB, Canada) in sheds with heat lamps overnight until they were 35 days old, when they were moved to wire dog kennels (each approximate 3 x 3 x 2 m). Ducks were fed ~ 250 g each day of 21% unmedicated Grower Crumbles ration (Hi-Pro Feeds Inc., Sherwood Park, AB, Canada). In late October 2011, the birds were moved to a private farm east of Edmonton for over-winter maintenance. Adult ducks were fed a 17% protein grower/finisher non-medicated ration (Hi-Pro Feeds Inc.) and well water was provided ad libitum.

3.2.2 Experimental Protocol

To test whether effects of exposure to OSPW differed depending on life stage, birds were exposed both as juveniles and as adults. In the first trial (September 2011), we randomly assigned juvenile birds into a control (n = 11) or treatment group (n = 25). Although we had intended for birds to remain in their experimental groups for the duration of the 2 year experiment, several birds shed their identification bands. In the second trial (July 2012), those that retained their ID remained in their original groups while those without bands were randomly re-assigned while equalizing sexes between control (n = 14) and treatment (n = 15) groups. Seven birds were re-assigned from the treatment to control group, and one from the control to treatment group (Table 3.1).

Table 3.1: Experimental design

	2011		2012	
	Control	**Treatment**	**Control**	**Treatment**
Male	6	13	8	8
Female	5	12	6	7
Total	11	25	14	15

Control groups were exposed to local well water obtained from each of the study areas, and treatment groups to OSPW obtained from the recycled water pond at Shell Canada's Muskeg River Mine (57°15'18.87"N, 111°29'59.69"W). In 2011, water was pumped directly from the pond into plastic Rubbermaid bins and transported back to the field site where it was also stored in those bins. In 2012, water was transported back to the Edmonton area and stored in 220 gallon water hauling tanks (Blaze Plastics Inc., AB, Canada) in the field.

Each exposure consisted of placing individual birds in 60 L plastic tubs containing approximately 15 L of either OSPW or control water. All exposures were 6 hours, except for the final exposure in trial 1, which was 8 hours. To ensure ducks were ingesting the water, they were offered approximately 100 mL of chopped greens (lettuce, spinach, chard, etc.) floating on the surface of the water several times during each exposure period. In trial 1, birds were exposed once weekly for three weeks. In trial 2, to emulate migratory bird stop-overs during spring and fall migration, birds were also exposed once weekly for three weeks, and following a 4 week period without exposure, were again exposed weekly for three weeks (Figure 3.1).

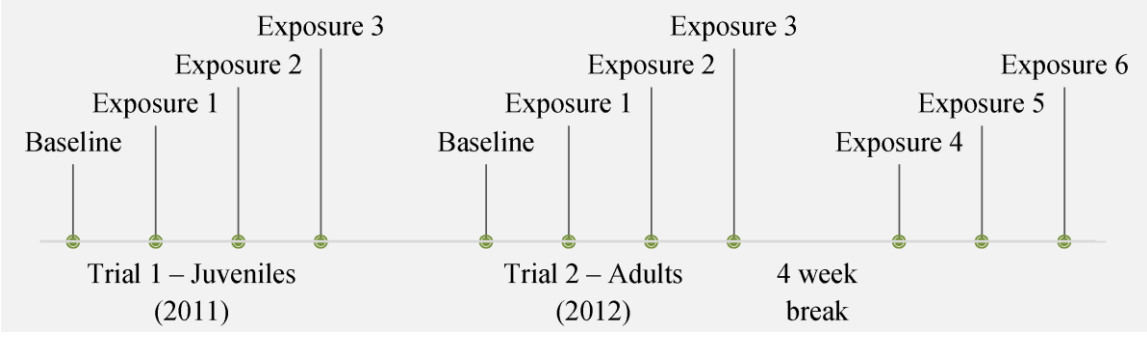

Figure 3.1: Timeline of exposure events

3.2.3 Sample Collection

In trial 2, we weighed birds before each exposure. Blood samples (1–4 mL, with total volume not exceeding 1% of body mass) were collected from the jugular or tarsal veins in juveniles and the tarsal or brachial veins in adults with 23–25 gauge needles (juveniles) or 22 gauge needles (adults). Samples were collected one week before trials 1 and 2 (baseline samples), and immediately following every exposure. Samples were immediately placed into heparinized vacutainers (Becton-Dickinson and Co, Franklin Lakes, NJ, USA) and stored in a cooler with icepacks during processing and transportation. In trial 1, we centrifuged whole blood for 5 minutes at 400 *g*, removed the plasma, and then stored it at -20 °C (Eppendorf 5415D, Eppendorf AG). In trial 2, we used portions of each blood sample for hematology (whole blood sample, hematocrit tubes and blood slides) before centrifuging the remainder at 1258 *g* for 6 minutes at 4 °C (Eppendorf 5810R, Eppendorf AG). Plasma was stored at -20 °C until analysis.

3.2.4 Sample Analysis

Control and OSPW samples from 2012 exposures were analyzed by ALS Environmental (Edmonton, AB, Canada) to quantify the concentrations of various oil sands compounds of importance; ALS reports from 2011 OSPW were provided by Shell Albian Sands (Table 3.2).

Plasma samples were submitted to Prairie Diagnostic Services (Western College of Veterinary Medicine, Saskatoon, SK, Canada) for biochemical, endocrinological, metal, and mineral evaluation (Table 3.2). The Avian Standard Chemistry panel included the following: sodium, potassium, chloride, bicarbonate, anion gap, calcium, phosphorous, glucose, cholesterol, GGT, GLDH, creatine kinase, AST, total protein, albumin, globulin, albumin: globulin ratio (A:G), uric acid, and bile acid. The endocrine panel measured levels of plasma triiodothyronine (T3), thyroxine (T4), and corticosterone. No samples were submitted for exposures 4 or 5 in the second trial. For the analysis of metals and minerals, we only used whole blood samples obtained from the final exposure period of the second trial. To increase blood volume for these analyses, we pooled samples within sex and experimental group to reduce 29 samples into 11, each composed of 2 or 3 birds.

The resulting panel measured levels of beryllium, magnesium, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, arsenic, selenium, strontium, molybdenum, cadmium, tin, antimony, barium, thallium, lead, and bismuth.

Blood samples were submitted to IDEXX Laboratories (Edmonton, AB, Canada), for comprehensive hematological analyses. Analysis included packed cell volume (PCV), total WBC (counted and calculated from blood smears), and differential WBC counts: heterophils, lymphocytes, monocytes, eosinophils, and basophils (Table 3.2). Hematology was only performed for the final three exposures of the second trial.

Table 3.2: Summary of diagnostic endpoints

Diagnostic	**Analysis**	**Samples**	**Measurements**		
Body condition		Trial 2	Weight		
Water	ALS Environmental	Trial 1 and 2	Carbon Metals Organics	Anions and Nutrients Physical	Hydrocarbons
Plasma Chemistry and Endocrinology	Prairie Diagnostic Services	All exposures except 4 and 5 of Trial 2	Sodium Potassium Chloride Bicarbonate Anion gap Calcium Phosphorous Glucose	Cholesterol GGT GLDH CK AST Total protein Albumin	Globulin A:G Uric acid Bile acid T3 T4 Corticosterone
Metals / Minerals		Final exposure of Trial 2	Beryllium Magnesium Vanadium Chromium Manganese Iron Cobalt	Nickel Copper Zinc Arsenic Selenium Strontium Molybdenum	Cadmium Tin Antimony Barium Thallium Lead Bismuth
Hematology	IDEXX Laboratories	Final 3 exposures of Trial 2	Hematocrit WBC Basophils	Eosinophils Heterophils	Lymphocytes Monocytes

3.2.5 Statistical Analysis

All statistics were carried out using R statistical software (R Core Team, 2013). We compared measures from control and treatment birds for each panel (biochemical, endocrinological, hematological, and body masses), using linear mixed effects models

(R-package "nlme"; Pinheiro et al., 2013). Eosinophil data were zero-inflated (74%) and so were converted to a binary variable and analyzed using logistic regression. All analyses were performed separately for juveniles and adults. We began with a saturated model containing sex, exposure, and experimental group as fixed effects, individual ID as a random effect, and all two-way interactions. If the residuals from initial models were heterogeneous, we selected different variance or auto-correlation structures and chose the model that produced the lowest AIC score. We refined models by applying backwards, stepwise likelihood ratio tests to remove all non-significant terms ($\alpha = 0.25$; Appendix I).

To accommodate multiple dependent variables, small sample sizes, and violations of normality and heterogeneity contained in our analysis of metals and minerals, we used a permutational multiple analysis of variance (perMANOVA; R-package "vegan"; Oksanen et al., 2013). A perMANOVA is a non-parametric, multivariate test applicable when the simultaneous responses of multiple potentially non-independent response variables have been measured from a multifactorial ANOVA design (Anderson, 2001). Current methods do not allow the use of this method with a repeated measures sampling design as is the case for the previous data set. We standardized data using z-scores (mean = 0, SD = 1) to account for variation in the detectability limits of different analytes. The multivariate model was conducted using Euclidean distances and included all response variables with both sex and experimental group as explanatory variables. Univariate models were conducted to aid interpretation using the same procedure as for the multivariate model.

To determine whether (1) juvenile mortalities and (2) the re-assignment of adult birds between experimental groups had an effect on overall results, we compared the number of mortalities between groups and the health values of birds that were designated as controls in both years with those so designated only in the first year. We examined the first effect with Fisher's exact test of independence and for the second we assigned a new binary factor to represent the re-assignment of experimental group and re-ran our models to evaluate its significance.

3.3 Results

3.3.1 Water Chemistry

In general, OSPW had greater concentrations of metalloids (e.g., Mg, K, and Ca), organic compounds (e.g., NAs, TOC, COD, 2-Fluorobiphenyl, *p*-Terphenyl d14 and F3) and ions (e.g., Cl^- and SO_4^{2-}), as well as lower concentrations of most inorganic measures (e.g., alkalinity, HCO_3, DIC, and conductivity (Table 3.3). Results were compared to water quality guidelines when available. OSPW concentrations of Al, Fe, Zn and phenols, control concentrations of HCO_3, and both control and OSPW concentrations of fluoride exceeded these guidelines (Table 3.3; CCME, 2013; USEPA, 1986, 2013; SETAC, 2004).

Table 3.3: Water chemistry of OSPW and control (well) water

Type	Analyte (mg/L unless otherwise noted)	Control	OSPW	Freshwater Quality Guidelines
Metals	Al	0.011–0.034	**0.81–8.85**	0.1 at pH ≥ 6.5[a]
	As	—	0.0029–0.0031	0.005[a]
	Ba	0.11–0.12	0.14–0.23	1[b]
	Be	—	0.000085–0.00042	0.0053[b]
	B	0.30–0.34	**1.86–2.03**	29[ST] or 1.5[LTa]
	Ca	4.39–4.59	23.7–27	
	Cr	—	0.0022–0.011	0.001–0.0089[a]
	Co	0.000085–0.0001	0.0024–0.0034	
	Cu	0.0018–0.00224	**0.00142–0.0043**	0.00039–0.0027[# a]
	Fe	0.075–0.087	**0.45–1.74**	0.3[a]
	Pb	0.00015–0.00021	0.001–0.0019	0.00022–0.004[# a]
	Li	0.078–0.08	0.15–0.17	
	Mg	0.3–0.31	13.2–15	
	Mn	0.00406–0.0057	0.045–0.11	0.1[b]
	Mo	0.0018–0.0019	0.046–0.058	0.073[a]
	Ni	—	0.00709–0.0095	0.019–0.11[#a]
	K	—	19.6–21.4	

	Se	—	0.00057–0.00074	0.001[a]
	Si	4.24–4.31	2.4–24.4	
	Ag	—	0.0000061–0.0000084	0.0001[a]
	Na	446–471	291–330	
	Sr	0.084–0.092	0.51–0.63	
	Tl	—	0.000012–0.000056	0.0008[a]
	Sn	—	0.00023–0.0003	
	Ti	0.0013	0.01–0.22	
	V	0.00011	0.0074–0.018	
	Zn	**0.035–0.036**	**0.092–0.34**	0.03[a]
Hydro-carbons (µg/L)	F2	—	1–1.52	
	F3	—	8.65–11	
	F4	—	0.32	
	2-Fluorobiphenyl (%)	85.9–86.1	81–126	
	Acenaphthene	—	0.24	5.8[a]
	p-Terphenyl d14 (%)	91.6–94.5	107–137.4	
Organics	Naphthenic Acids	1	**24.4–30.9**	30[c]
	BOD	2.7–14.9	12.9–12.9	25[c]
	COD	10–16	148–174	200[c]
	Oil and Grease	—	1–2.2	197[a]
	Phenols (4AAP)	0.0012–0.0021	0.0065–0.0196	0.004[a]
Carbon	DIC	230–242	45.1–84	
	DOC	**8.7–10.1**	**40.5–59.5**	6.5–9.5[a]
	Total Organic Carbon	8.8–8.9	43.7–61.8	
Physical	Color (C.U.)	5.4–12.4	22.1–114	
	TDS	1080–1090	1050–1130	1340[d]
	TSS	4–23	7–72	
	Turbidity (NTU)	2.63–6.07	27.9–532	
Anions and Nutrients	Alkalinity	1010–1040	336–342	20[b]
	Ammonia	0.26	0.0261–0.25	0.029–0.343[a]
	HCO_3	1060–1180	106–410	500[c]
	CO_3	42.9–88.2	24–149	
	Chloride	5.9–6.01	155–224	230[b]
	Conductivity (uS/cm)	1760–1780	1510–1680	

	Fluoride	0.58–0.59	2.95–3.54	0.12[a]
	Hardness	12.1–12.6	112–119	50–380[d]
	Ion Balance (%)	96.4–102	91–114	
	Nitrate and Nitrite	0.016	0.015–0.16	
	Nitrate	0.0095	0.092–0.096	550[ST] or 13[LTa]
	Nitrite	0.0063	0.0035–0.06	0.12[a]
	pH	8.66–8.98	**8.34–10.14**	6.5–9.0[a]
	Phosphorus	**0.045–0.14**	0.039–0.18	0.004[a]
	SO_4	0.096	172–189	
	Sulphide	0.0034	0.0046–0.0053	
	Total Kjeldahl Nitrogen	0.48–0.54	0.81–1.7	

Note: Bolded values exceeded water quality guidelines. "—" indicates the value was below the detection limit; #, interval depends on hardness; ST/LT, Short/Long term; BOD/COD, biochemical/chemical oxygen demand; DIC/DOC , dissolved inorganic/organic carbon; TDS/TSS, total dissolved/suspended solids; a (CCME, 2013); b (USEPA, 2013); c (Allen, 2008); d (SETAC, 2004).

3.3.2 Body Condition and Survival

Adult weights did not differ between experimental groups (Table 3.4; $F_{1,24} = 2.1$, $p > 0.05$), nor did survival (3 controls and 4 treatment; $p > 0.05$). Seven birds died over the course of the two trials, but their deaths were attributed to intra-specific aggression in five cases, predation in one case, and in the last case, of unknown causes. All remaining birds had developed moderate to severe pododermatitis by the end of the second trial, which is common in domestic ducks and was attributed to over-wintering housing conditions (Table 3.5; Ritchie et al., 1994). Re-assignment of experimental groups upon adulthood was not a significant predictor in 84% of models ($F_{1,24\text{-}26} > 3.2$, $p > 0.089$), nor did it change the significance of other terms in the remaining models in which it was retained.

Table 3.4: Mean (± SE) weights from adult ducks exposed to OSPW and control water

Baseline		**Final**		
Control	**OSPW**	**Control**	**OSPW**	**Effect Size Final (%)**
4.25 ± 0.1	4.19 ± 0.1	4.49 ± 0.2	4.29 ± 0.1	-4.36

Note: All units in are in kg

Table 3.5: Pododermatitis classification system

Score	Category	Description or ranking criteria
0	Normal / None	No indication of inflammation
1	Affected / Marked	Evidence of inflammation: ulcer, swelling of joints or soft tissue, rough, proliferative, hyper-keratotic epithelium

3.3.3 Clinical Biochemistry

Summary statistics for biochemical and endocrinological analytes are presented in Table 3.6; significant model results are discussed below. Based on the analyses of electrolytes, there were no differences between control and treatment groups for sodium, chloride, or anion gap in juvenile or adult birds ($F_{1,25\text{-}113} < 2.7$, $p > 0.05$ for each). However, potassium levels in juveniles were 20% greater in treatment birds than controls, but only following the final exposure (Figure 3.2; exposure × experimental group: $\beta = 0.19 \pm 0.09$, DF = 104, $p = 0.026$). In adults, baseline potassium levels were 12% higher than controls, but this difference decreased to 5% by the final exposure (Figure 3.2; exposure × experimental group: $\beta = 0.053 \pm 0.03$, DF = 113, $p = 0.037$). Also as an interacting effect (exposure × experimental group), juveniles exposed to OSPW had similar bicarbonate levels at baseline but 6% lower than controls following the final exposure (Figure 2.2; $\beta = -0.58 \pm 0.3$, DF = 104, $p = 0.04$). In adults they were 7% less than controls at baseline, and 5% greater by the end of the trial (Figure 3.2; $\beta = 0.36 \pm 0.2$, DF = 113, $p = 0.03$).

Through analyses of biochemical metabolites and enzymes we found no differences between experimental groups in calcium, phosphorus, total protein, albumin, A:G ratio, glucose, AST, GLDH, or CK in either juveniles or adults, nor for juvenile levels of GGT, globulins, uric or bile acids, nor adult cholesterol levels ($F_{1,25–114} < 5.3$, $p > 0.05$ for each). We found differential effects of experimental group dependent on exposure (exposure × experimental group) for cholesterol in juveniles ($\beta = -0.12 \pm 0.05$, DF = 104, $p = 0.03$), and uric acid in adults ($\beta = 6.8 \pm 2.2$, DF = 112, p = 0.0024). Concentrations in both were higher in treatment birds at baseline (6%, 47% respectively), but this difference reversed or decreased by the final exposure (Figure 3.3, S3; -5%, 8% respectively).

In adults, we also found differential effects of experimental group by sex (sex × experimental group) for bile acids (β = -5.8 ± 1.9, DF = 25, p = 0.0061) and globulins (β = -4.4 ± 1.8, DF = 25, p = 0.02). Specifically, females exposed to OSPW had higher levels of bile acids (37%) and globulins (17%) than controls, whereas differences between experimental groups were not as pronounced for males (Figure 3.3; 12% and 7% respectively). Finally, relative to controls, adults exposed to OSPW had 7% lower GGT (Figure 3.4; β = -0.60 ± 0.27, DF = 26, p = 0.03) levels.

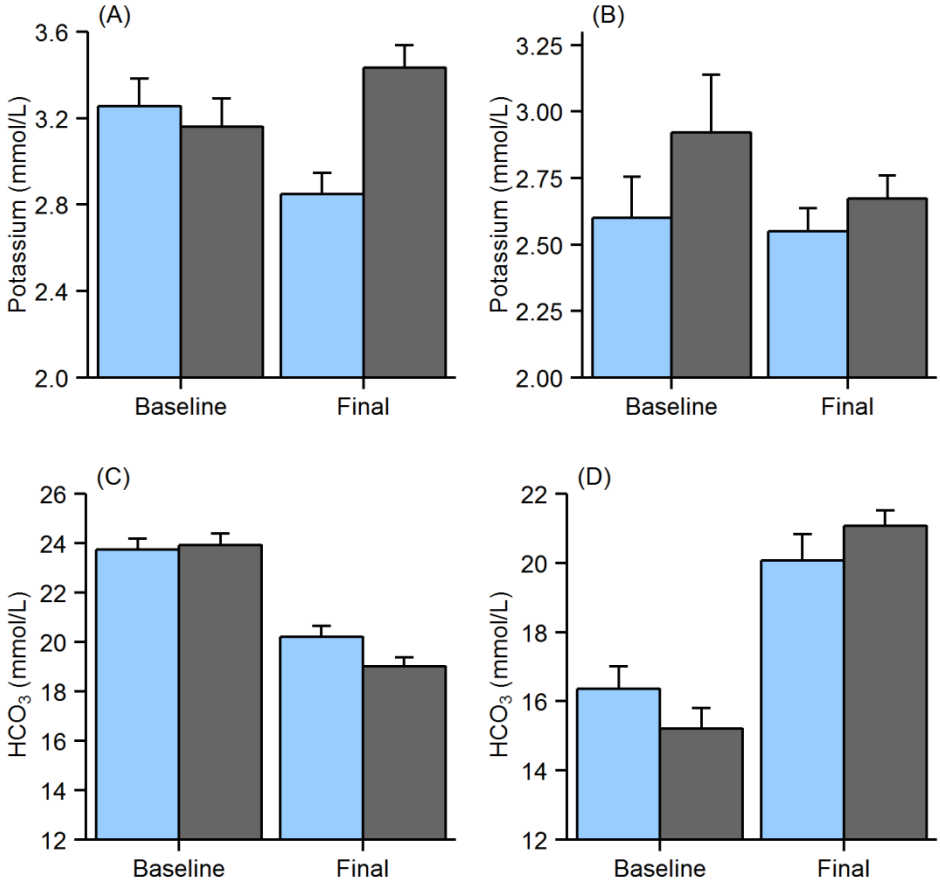

Figure 3.2: Mean (± SE) concentrations of plasma potassium and bicarbonate in baseline samples and following the final exposure from juvenile (A and C) and adult (B and D) ducks exposed to control and OSPW (blue and grey bars, respectively).

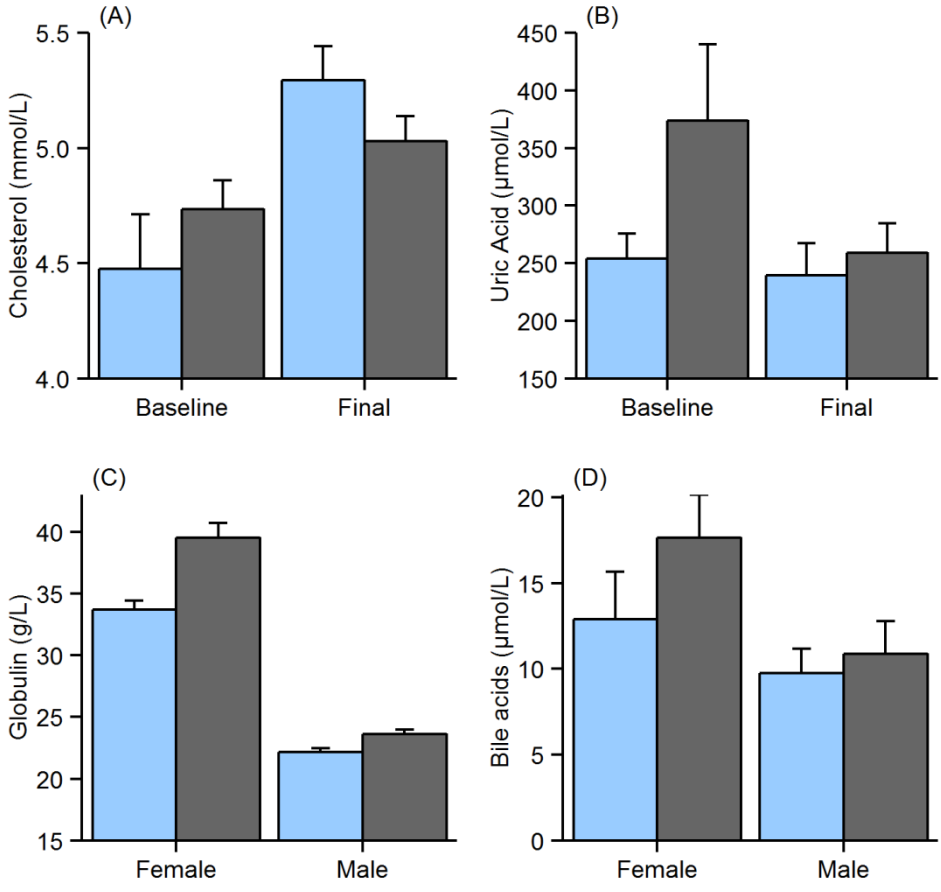

Figure 3.3: Mean (± SE) concentrations of (A) cholesterol in juvenile and (B) uric acid in adult baseline samples and following the final exposure. Mean (± SE) concentrations (C) globulin and (D) bile acid levels from male and female adult ducks exposed to control and OSPW (blue and grey bars, respectively).

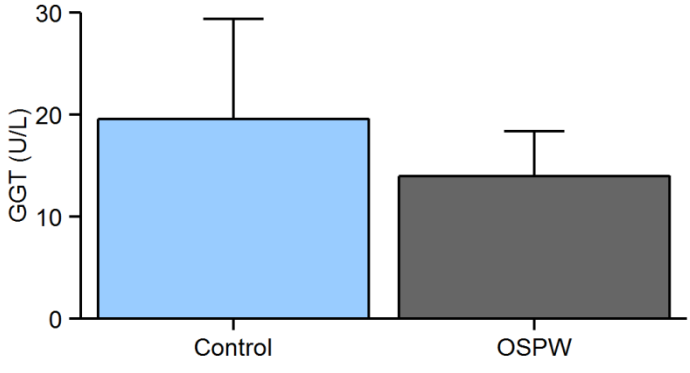

Figure 3.4: Mean (± SE) concentrations of GGT in adult ducks exposed to control and OSPW (blue and grey bars, respectively).

3.3.4 Endocrinology

There were no differences between experimental groups for T3 or T4 in either age group, nor for juvenile corticosterone or adult thyroid levels ($F_{1,\,25–107} < 3.6$, $p > 0.05$ for each). In juvenile ducks we found that males exposed to OSPW had a 26% higher ratio of thyroid hormones (T3/T4), whereas treatment females were 14% lower than controls (Figure 3.5; sex × experimental group: β = 0.066 ± 0.02, DF = 32, $p = 0.016$).

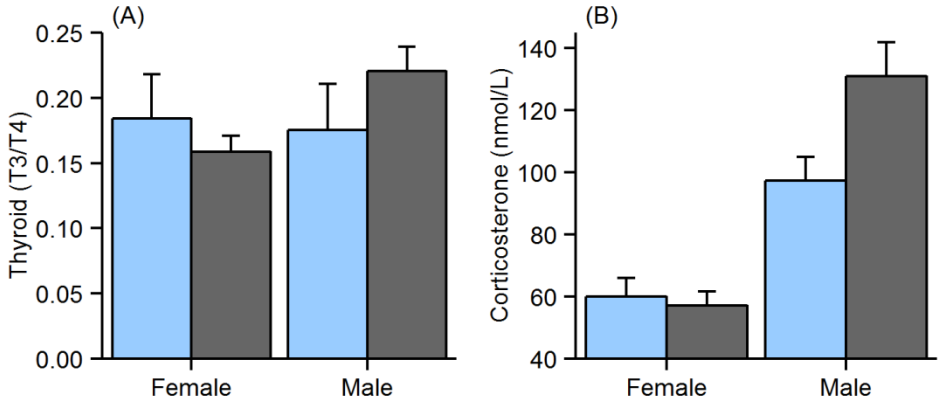

Figure 3.5: Mean (± SE) concentrations of (A) the thyroid hormone ratio and (B) corticosterone, from male and female ducks exposed to control and OSPW (blue and grey bars, respectively).

Table 3.6: Mean (± SE) plasma biochemistry and endocrinology concentrations from baselines samples and following the final exposure periods in juvenile and adult ducks exposed to OSPW and control water

	Juvenile					**Adult**					
	Baseline		**Final**			**Baseline**		**Final**			
Analyte	Control	OSPW	Control	OSPW	ES	Control	OSPW	Control	OSPW	ES	RR
Na (mmol/L)	141.8 ± 2.07	145.1 ± 0.4	147.2 ± 0.3	147.3 ± 0.4	0.1	145 ± 1.14	146.3 ± 0.7	145.1 ± 0.5	146.9 ± 0.6	1	130–160[a]
K (mmol/L)	3.25 ± 0.13	3.16 ± 0.13	2.85 ± 0.1	3.43 ± 0.1	20	2.6 ± 0.16	2.92 ± 0.2	2.55 ± 0.1	2.67 ± 0.1	5	2–4[a]
Cl (mmol/L)	99.18 ± 1.5	101.2 ± 0.2	103.5 ± 0.3	103.7 ± 0.4	0.2	102.5 ± 0.9	102.9 ± 0.7	103.7 ± 0.7	105 ± 0.7	1	100–120[a]
HCO_3 (mmol/L)	23.7 ± 0.4	23.9 ± 0.5	20.2 ± 0.4	19 ± 0.4	-6	16.4 ± 0.7	15.2 ± 0.6	20.1 ± 0.8	21.1 ± 0.5	5	20–30[a]
Anion gap	22.3 ± 0.7	23.08 ± 0.5	26.6 ± 0.6	28.2 ± 0.4	6	28.9 ± 1.09	31.2 ± 1.15	24 ± 1	23.6 ± 0.7	-2	15[b]
Ca (mmol/L)	2.807 ± 0.08	2.881 ± 0.02	2.923 ± 0.04	2.933 ± 0.02	0.3	4.105 ± 0.5	4.785 ± 0.8	3.81 ± 0.5	4.634 ± 0.8	22	2–3[c]
P (mmol/L)	2.311 ± 0.08	2.388 ± 0.04	2.676 ± 0.04	2.585 ± 0.04	-3	1.445 ± 0.2	1.823 ± 0.2	1.631 ± 0.2	2.068 ± 0.3	27	1–3[c]
Glucose (mmol/L)	9.5 ± 0.4	9.52 ± 0.15	9.84 ± 0.3	9.61 ± 0.15	-2	8.96 ± 0.3	9.58 ± 0.5	9.27 ± 0.7	8.71 ± 0.9	-6	7–18[c]
Cholesterol (mmol/L)	4.475 ± 0.2	4.735 ± 0.13	5.295 ± 0.15	5.03 ± 0.11	-5	3.346 ± 0.3	3.103 ± 0.4	4.714 ± 0.4	4.189 ± 0.3	- 1	3–6[c]
Uric acid (µmol/L)	249.9 ± 28.9	222.8 ± 22.5	347.6 ± 44.7	299.3 ± 14.8	-14	254.1 ± 21.5	373.5 ± 66.4	239.4 ± 27.8	259.1 ± 25.7	8	119–701[c]

Bile Acid (µmol/L)	15 ± 3.6	18.9 ± 2.9	5.9 ± 0.7	6.4 ± 0.6	9	30.3 ± 3.7	33.2 ± 5.06	6.5 ± 1.4	9.1 ± 0.9	41	22–82[c]
GGT (U/L)	2.5 ± 0.16	2.7 ± 0.11	2.5 ± 0.3	2.8 ± 0.2	10	9.6 ± 3.5	7.87 ± 3.03	29.5 ± 19.3	20 ± 8.2	-32	0–10[d]
GLDH (U/L)	1.8 ± 0.3	3 ± 0.4	1.8 ± 0.3	2.3 ± 0.2	25	1.7 ± 0.5	4.4 ± 1.9	1 ± 0.2	1.4 ± 0.3	40	< 10[a]
CK (U/L)	667.5 ± 74.004	642 ± 46.009	1268.6 ± 183.7	1179.5 ± 133.7	-7	744.9 ± 103.4	651.6 ± 60.7	405.4 ± 45.9	476.5 ± 81.3	18	165–378[c]
AST (U/L)	6.3 ± 0.8	7.1 ± 0.9	7.7 ± 1.2	9.2 ± 0.9	19	22.2 ± 2.9	36.07 ± 8.12	8.1 ± 0.9	10.1 ± 0.9	25	12–73[c]
Total Protein (g/L)	32.5 ± 1.8	35.6 ± 0.5	36 ± 0.6	36.8 ± 0.5	2	42.6 ± 2.8	47.7 ± 3.08	45.1 ± 2.2	47.5 ± 2.7	5	35–55[c]
Albumin (g/L)	13.8 ± 0.9	14.9 ± 0.3	17.4 ± 0.3	17.2 ± 0.3	-1	16.5 ± 1	16.3 ± 0.9	17.6 ± 0.5	18.3 ± 0.9	4	17–22[c]
Globulin (g/L)	18.6 ± 1.06	20.6 ± 0.4	18.6 ± 0.3	19.6 ± 0.4	6	26.14 ± 1.9	31.3 ± 2.7	27.4 ± 1.8	29.1 ± 1.8	6	35–60[c]
A:G ratio	0.7 ± 0.03	0.7 ± 0.015	0.9 ± 0.01	0.9 ± 0.02	-6	0.6 ± 0.02	0.6 ± 0.04	0.7 ± 0.03	0.6 ± 0.02	-4	1–2[c]
Corticosterone (nmol/L)	51.302 ± 8.6	57.339 ± 8.8	65.221 ± 5.3	52.703 ± 5.3	-19	100.934 ± 11.15	117.868 ± 19.4	74.903 ± 8.5	100.671 ± 20.2	34	228–1286[e]
T3 (nmol/L)	1.529 ± 0.2	1.879 ± 0.12	1.86 ± 0.1	2.047 ± 0.1	10	1.371 ± 0.17	1.221 ± 0.16	2.007 ± 0.16	1.597 ± 0.14	-20	0.2–2[e]
T4 (nmol/L)	8.27 ± 1	11.02 ± 1.5	14.738 ± 1.4	14.498 ± 1.12	-2	9.348 ± 1.3	10.759 ± 1.8	7.869 ± 1.6	10.941 ± 1.7	39	8–81[e]
T3/T4	0.272 ± 0.09	0.236 ± 0.03	0.135 ± 0.01	0.158 ± 0.013	16	0.147 ± 0.03	0.133 ± 0.02	0.397 ± 0.1	0.264 ± 0.1	-34	

Note: Effect size (ES) is described as the percentage difference relative to controls ([(OSPW - Control) / Control]*100). Reference ranges or means are examples and do not show the full range of levels present in the literature. Six cases were removed from T3 due to inadequate sample volume, and two samples were lost in the final exposure of 2011. a (Thrall, 2012); b (Mayer and Donnelly, 2010); c (Fudge, 2003); d (Harr, 2002); e (Prairie Diagnostic Services, 2012).

3.3.5 Hematology

There were no differences for PCV, total WBC, heterophils, lymphocytes, eosinophils, or basophils (Table 3.7; F1, 25–56 < 3.5, p > 0.05 for each). Monocytes were 53% higher in control birds following exposure 4, and 10% higher in treatment birds following the last exposure (Figure 3.6; exposure × experimental group: $\beta = 0.24 \pm 0.09$, DF = 54, $p = 0.0016$).

Table 3.7: Mean (± SE) levels of several hematology parameters from the final exposure period in adult ducks exposed to OSPW and control water

Analyte	**Control (n = 14)**	**OSPW (n = 15)**	**Effect Size (%)**	**Reference Means[a]**
PCV (%)	43.6 ± 1	43.5 ± 1.1	-0.4	36.15
WBC ($x10^9$/L)	19.4 ± 2.3	15.7 ± 1.4	-19	19.5
Basophils ($x10^9$/L)	0.342 ± 0.1	0.307 ± 0.1	-10	1.13
Eosinophils ($x10^9$/L)	0.365 ± 0.1	0.078 ± 0.03	-79	0.12
Heterophils ($x10^9$/L)	13.87 ± 1.9	11.637 ± 1.3	-16	12.11
Lymphocytes ($x10^9$/L)	4.251 ± 0.4	3.176 ± 0.4	-25	6.035
Monocytes ($x10^9$/L)	0.476 ± 0.1	0.522 ± 0.1	10	0.12

Note: Effect size is described as the percentage difference relative to control ([(OSPW - Control) / Control]*100). a (Hatipoğlu and Bağci, 1996).

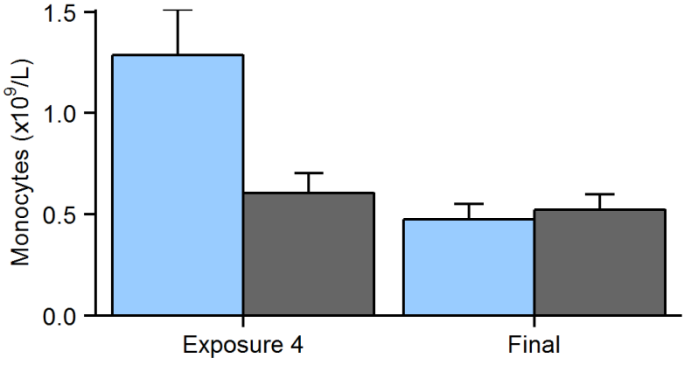

Figure 3.6: Mean (± SE) concentrations of monocytes following the fourth and final exposures in adult ducks exposed to control and OSPW (blue and grey bars, respectively).

3.3.6 Metals and Minerals

The multivariate analysis of metals and minerals did not reveal any differences between experimental groups (Table 3.8; $F_1 = 0.96$, $p = 0.47$). However, univariate models showed that treatment birds had 244% higher levels of vanadium than controls (Figure 3.7; $F_1 = 8.33$, $p = 0.032$), and molybdenum was 28% higher in female birds treated with OSPW as compared to males, where levels were 20% less than controls (Figure 3.7; sex × experimental group: $F_1 = 7.057$, $p = 0.048$).

Table 3.8: Mean (± SE) whole blood metal and mineral concentrations from the final exposure period in adult ducks exposed to OSPW and control water

Analyte (µg/dL)	Control (n = 5)	OSPW (n = 6)	Effect Size (%)
As	0.62 ± 0.3	0.42 ± 0.2	-32
Ba	11 ± 4.8	25.4 ± 13.8	131
Cd	0.033 ± 0.015	0.0492 ± 0.003	49
Co	0.204 ± 0.03	0.212 ± 0.02	4
Cr	5.74 ± 0.6	7.33 ± 0.5	28
Cu	33.2 ± 4.4	42.5 ± 5.8	28
Fe	38096 ± 2941.8	36765 ± 2126	-3
Mg	6348 ± 533.8	6231.67 ± 185.1	-2
Mn	4.36 ± 1.9	5.783 ± 2.3	33
Mo	13.38 ± 1.3	13 ± 0.7	-3
Ni	2.32 ± 0.5	2.4 ± 0.4	3
Pb	2.76 ± 0.8	4.55 ± 1.1	65
Sb	0.143 ± 0.04	0.13 ± 0.005	-9
Se	30.8 ± 3.7	31.5 ± 0.9	2
Sn	1.135 ± 0.16	1.549 ± 0.4	36
Sr	9.2 ± 3.4	13.27 ± 6.6	44
Tl	0.0292 ± 0.004	0.0285 ± 0.009	-2
V	1.98 ± 0.8	6.79 ± 1.5	244
Zn	339.8 ± 51.7	361 ± 46.7	6

Note: To increase volume to meet analytical requirements samples were pooled within sex and experimental group. Effect size is described as the percentage difference relative to control ([(OSPW - Control) / Control]*100). Bismuth and beryllium were removed as they were below detection limits.

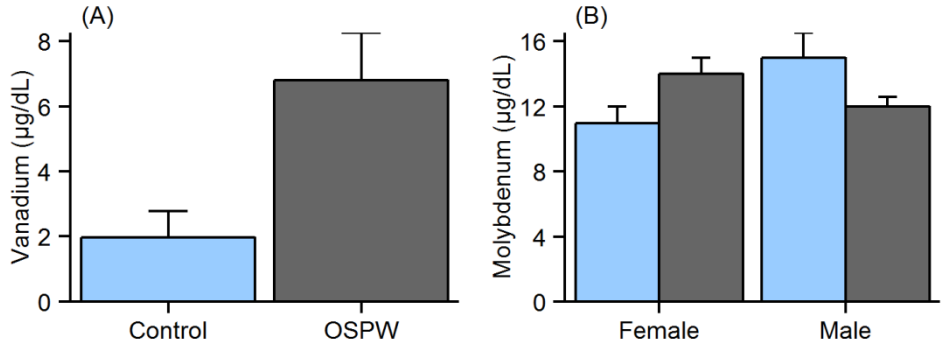

Figure 3.7: Mean (± SE) whole blood concentrations of (A) vanadium, and (B) male and female levels of molybdenum from adult ducks exposed to control and OSPW (blue and grey bars, respectively).

3.4 Discussion

The objective of this study was to determine whether repeated, short-term exposure to OSPW would adversely affect duck health as determined by measurements of physiological indicators. Using captive pekin ducks as a model, we did not find consistent or strong evidence of such effects based on measures of body mass, biochemistry, endocrinology, hematology, metal or mineral levels. This conclusion is based not only on statistical analyses, but also on the relation to reference values of the few variables that exhibited differences. Reference intervals, or reference ranges, are critical in interpreting the biological relevance of differences in health variables, as many biological measures will vary substantially depending on the age, sex, and reproductive status of the source population (Thrall, 2012). While specific intervals for pekin ducks were not available for all analytes, comparisons with those of other avian species, preferably those with a close taxonomic relationship, are routinely used as guidelines in medical and health assessments and interpretations. Although there were some statistically significant differences between control and treatment birds, we found that mean values for both groups were within the most appropriate reference intervals that could be compiled for pekin ducks (Hatipoğlu and Bağci, 1996; Pedersoli et al., 1989; Prairie Diagnostic Services, 2012; Spano et al., 1987), mallards (Harr, 2002; ISIS, 2002; Tully et al., 2009), or general bird ranges (Mayer and Donnelly, 2010; Thrall, 2012).

We found no evidence that exposure to OSPW influenced the body mass of adult birds. Oil toxicity's reduction of growth and weight in birds is well established in the

literature (Gurney et al., 2005; Szaro et al., 1981, 1978), although mechanisms are not described. Direct comparisons between the literature and the present study are not relevant due to differences in the type of oil used, the exposure conditions, and biological differences between species, and even sub-species (Leighton, 1993). For example, *A. platyrhynchos* weigh 1.2 kg on average (Ritchie et al.,1994), while *A. platyrhynchos domesticas* have been domesticated and bred for meat production, and weigh 3.2 kg on average (Cornell University, 2008).

Clinical biochemistry analysis included evaluation of plasma electrolytes, metabolites, and enzymes; for ease of interpretation these will be described according to their effects on the body systems. We found no evidence of toxicity specific to glucose metabolism (glucose), muscle injury (creatine kinase) or calcium regulation.

Electrolyte and acid–base balance analytes were unaffected (sodium, chloride, and anion gap) with the exception of potassium and bicarbonate. In juvenile treated birds, we found that differences from controls increased with exposure whereas in adults differences were most pronounced in baseline samples but declined thereafter. Elevated potassium can be associated with severe renal disease (Thrall, 2012), but varying results have been found in previous studies. Decreases in potassium were found in adult pigeon guillemots sampled in sites affected by the Exxon-Valdez (Golet et al., 2002) while other studies have found no effect in mallard ducklings (Szaro et al., 1978) or adults (Coon and Dieter, 1981) fed crude oil. In our study, we did not expect differences to be present in baseline samples if they occurred because of experimental manipulation. Given that potassium values were well within reference intervals for similar waterfowl species, we do not believe differences are of biological concern. Similarly, bicarbonate levels following final exposures were higher in controls for juvenile birds and higher in treated birds for adults; however, neither difference is severe enough to indicate metabolic acidosis (decreased) or alkalosis (increased).

Biochemical evaluation of the kidney found no evidence of toxicity for the majority of analytes (sodium or phosphorus), with the exception of potassium (as above) and uric acid. Uric acid levels in adults were higher in treated birds at baseline, but this difference decreased with repeated exposure. Elevated uric acid concentrations can be indicative of kidney disease (Ritchie et al., 1994) although its diagnostic importance in

birds is debated (Fudge, 1999). Oil-induced changes to uric acid concentrations are not well described, although one study found elevated concentrations in oiled female mallards both 5 days and 6 months post oiling, while no effect was seen in males (Huffman, 1999). Other studies have found no such differences in pigeon guillemots (Seiser et al., 2000), or mallards exposed to crude oil (Rattner, 1981). In our study, both experimental groups had mean uric acid levels within reference intervals, which does not indicate the presence of renal damage.

Biochemical evaluation of the birds' livers revealed no effect on several compounds that are increased during hepatic damage (AST or GLDH); though cholesterol levels in juveniles and GGT in adults exposed to OSPW were lower than controls following the final exposure. Previous work has shown decreased (Eastin and Rattner, 1982), increased (Newman et al., 2004), and unchanged (Szaro et al., 1981, 1978) cholesterol in response to oil exposure. Decreased GGT activity has been reported following fuel oil exposure in female yellow-legged gulls (*Larus michahellis*), which, researchers hypothesized, may be attributable to birds' reduced ability to increase GGT in response to a physiological condition, such as egg production (Alonso-Alvarez et al., 2007). However, we saw no such sex difference and GGT is primarily a non-parametric analyte, whereby increases are more significant than decreases (Fudge, 1999). Bile acids were also higher in adult females exposed to OSPW. Increased bile acids are both sensitive and specific to hepatic disease but in concentrations much higher than seen in this study (Fudge, 1999). Few studies have reported on bile acids following oil exposure. One study found no effect in pigeon guillemot (*Cepphus columba*) sampled following the Exxon Valdez (Golet et al., 2002), and the other, similar to ours, found statistical differences which were not supported by biological reference intervals (Seiser et al., 2000).

Evaluation of plasma proteins was not supportive of toxic effects for albumin, total proteins, or the A:G ratio. In adults, female treatment birds had higher globulins compared to controls. Elevated globulins, which are comprised partially of an antibody response to infection, are commonly seen with inflammation (e.g., pododermatitis), as well as preceding egg production in females (Thrall, 2012) both of which are possible confounding factors in our study.

Laboratory evaluation of hormone levels revealed no differences between experimental groups for T3 or T4. We did find a higher thyroid ratio in juvenile males, and higher corticosterone in adult males exposed to OSPW. Thyroid hormones are often measured in toxicological studies as they control critical physiological processes including basal metabolic rate, thermoregulation, growth, and reproduction (Fudge, 1999). Alterations to these thyroid hormones have been observed with controlled exposure to crude oils although effects vary; some studies have described increases in corticosterone, or T4, while others described no endocrine related changes (Leighton, 1993). A series of comparable field experiments on the effects of OSPW in tree swallows did find elevated plasma T3 and T4 (Gentes et al., 2007a), as well as elevated corticosterone in males nestlings on one of two experimental wetlands (Harms et al., 2010). Methodological differences limit the comparability between studies, namely study species, age of birds, and exposure type and duration. The increased corticosterone in the treated males may indicate that they responded to the difference in water quality. A study of longer-term exposure and chronic stress, such as could be determined from feather corticosterone analysis would be required to provide more compelling evidence of increased stress than is possible through this short term measure of plasma corticosterone. Additionally, other hormones, such as those associated with reproduction (e.g., estradiol, vitellogenin, etc.) may have elicited stronger responses.

No indication of toxicity was apparent in blood analysis (PCV, total or differential WBC's). Hematological evaluations have been used to identify oil induced toxicities such as hemolytic anemia (Leighton et al., 1983), as well as secondary effects such as stress, infection, and inflammation (Briggs et al., 1996). We found that monocyte numbers were lower in treatment birds following the fourth exposure, but comparable by the final exposure. Little evidence of oil-induced changes specific to monocytes have been reported (Newman et al., 1999), nor was the mean value of treated birds low enough to be of clinical concern.

Analysis of metals and minerals included a wide range of elements, many of which are listed by the USEPA as priority pollutants (USEPA, 2013). We found higher levels of vanadium in all treatment birds, and higher molybdenum in female treatment birds. Vanadium is a metal found in high concentrations in petroleum (Barceloux, 1999) yet

little is known about its toxicity to vertebrates. Previous work postulates that low level exposure is not likely to be acutely toxic to wildlife (Rattner et al., 2006), which is likely because at low levels, vanadium may be essential to birds; deficiencies have been linked to impairment of growth and reproduction as well as biochemical abnormalities (Barceloux, 1999). Attempts to use liver and kidney concentrations of vanadium in oiled seabirds as a biomarker for exposure revealed no differences from control birds (Kammerer et al., 2004), although another study found that vanadium accumulation in wild birds in japan was reflective of other types of environmental contamination (Mochizuki et al. 1999). In the current context at the levels observed, we suggest it therefore could be a biomarker of exposure, but likely not toxicity. Contrastingly, molybdenum is a naturally occurring essential micronutrient, but anthropogenic activities such as combustion of fossil fuels have contributed to its contamination in the environment (Eisler, 2000). Molybdenum is relatively non-toxic, although it has been shown to adversely affect growth, reproduction, and survival with high dietary concentrations ranging from 200 to 6,000 mg/kg (Eisler, 2000). Although there are no available reference intervals for the metal and mineral in avian blood samples, there were low levels of vanadium and molybdenum in both treatment (V = 0.0074 mg/L; Mo = 0.058 mg/L) and control (V = 0.0001 mg/L; Mo = 0.0018 mg/L) water.

While few toxic effects from short-term exposure to OSPW were identified in this study, there are important considerations for exposure of birds to oil sands process-affected water ponds. There is huge variation in these ponds; they vary in terms of their size, chemical constituents, toxicity, accessibility, and attractiveness to birds. Following bitumen extraction, remaining liquid waste is pumped to the tailings disposal site. As fresh tailings are delivered into ponds, they spontaneously divide into 3 layers: solids that settle to become mature fine tailings (MFT), suspended fine particles such as silt and clay, and a water layer available for recycling, as well as residual bitumen mats that tend to accumulate at the edges of pond surfaces (Devenny, 2010). The water layer is extracted and contained in recycled water ponds until its reuse in the bitumen extraction process. This recycled water generally does not contain visible bitumen, but each time the water goes through the extraction process, its chemistry changes: for example, dissolved salts accumulate (Allen, 2008; Devenny, 2010).

The toxicity of OSPW varies considerably and is a complex interaction between the compounds present in the water, the type, and age of the pond, as well as any remediation strategies that are occurring (Allen, 2008). Our research investigated the effects of recycled process-affected water on birds, while early toxicological research focused mainly on the acute toxicity PAHs and NAs to aquatic organisms. Naphthenic acids in particular have been the focus of a large body of research and have shown a range of effects in fish (e.g., Kavanagh et al., 2012; MacDonald et al., 2013; Peters et al., 2007), amphibians (e.g., Smits et al., 2012), and mammals (e.g., Rogers et al., 2002). Only one study to date has examined the effects of NAs in birds. Gentes et al., (2007b) found that a range of health variables were unchanged in experimentally dosed, wild tree swallow nestlings, exposed throughout most of their nestling period, although an increase in liver extramedullary erythropoiesis was noted. High concentrations of trace metals, ammonia, and total dissolved solids are also possible causes of concern (Allen, 2008), as well as the potential for the chronic effects of OSPW on aquatic and terrestrial organisms inhabiting reclaimed wetlands (Allen, 2008). Due to the range of ponds and toxicities currently on the landscape, our work cannot rule out the possibility of adverse health effects on birds exposed to OSPW obtained from other sources. Further toxicity testing on a wider range of process-affected water ponds, perhaps in a gradient of those considered least to most toxic would be a logical next step. Testing of this nature would also facilitate the development of potential effect levels (PELs) for the various contaminants present in OSPW.

Future studies could also account for the different ways in which wild birds interact with their environment that were not replicated in our exposures. Birds often land to feed on process-affected water ponds, creating additional pathways for contaminant exposure because bioaccumulation of toxins in sediment (Timoney and Lee, 2011), plants (Xu et al., 1997), and aquatic invertebrates (Brua et al., 2004) is likely to occur. Ingestion of grit in mallards has also shown to be a significant route of contaminant exposure for NAs, and oil and grease (King and Bendell-Young, 2000). There are also species-specific differences in the ways birds interact with their environment. For example, birds that feed on the water's surface interact differently with the pond than those that dive to feed.

Since we did not replicate all possible exposure routes, different species of wild birds may experience adverse health effects when they spend time on OSPW ponds.

While this study specifically emulated migratory bird interactions with the oil sands, resident birds may be more susceptible to oil sands contaminants. For example, recent work has shown that water bird eggs collected downstream of the oil sands contain high levels of mercury, and more specifically, historical data shows that mercury egg burdens in California and Ring-billed gulls (*Larus californicus* and *delawarensis*) have increased significantly since the first years of sampling (1977 and 2009 respectively; Hebert et al., 2013, 2011). As well, there could be additional health effects in birds not measurable by blood analyses, or those that require a longer duration of exposure to manifest at detectable levels. For example, multi-year examination of tree swallows nesting on reclaimed wetlands has shown potential for negative reproductive effects through reduced brood sizes, increased nestling mortality, and reduced fledgling success (Gentes et al., 2006). Waterfowl may also be sensitive to such effects, especially for those that nest on or in proximity to process-affected water ponds.

The negligible adverse physiologic effects documented in this study are in contrast to a large body of literature detailing toxic effects of bitumen and conventional oils on birds. If further research supports our findings and can provide a greater understanding of the risks ponds pose to waterfowl, there are important implications for a holistic approach to bird protection in the oil sands. In particular, results consistent with our findings would encourage segregation of pond constituents (e.g., with technologies such as booming and skimming) and encourage smaller-scale deterrent strategies targeting sites containing the more toxic derivatives of the oil sands extraction processes. This approach could increase deterrent efficacy where it is most needed, reduce the tendency for birds to habituate, and reduce the exposure of humans and wildlife to high levels of noise pollution (St. Clair et al., 2013).

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Chapter 4: General Discussion

The oil sands of northern Alberta are a classic example of how industrial by-products may pose threats to wildlife and their habitat. Of particular concern, are the health implications associated with avian contact with the toxic constituents of tailings ponds. Since the industry development in the late 1970's, concerns over waterfowl landing on tailings ponds have been voiced, yet very little research has been conducted on the subject. To address this knowledge gap, we first reviewed the documented toxicological effects that process-affected water can have on birds. We found a bias towards studies on tree swallows nesting on reclaimed wetlands. These studies have shown evidence of immunotoxicity, disruptions to thyroid hormones, reduced reproductive performance, and increased EROD activity. Another study on breeding birds on oil sands based wetlands showed an overall reduction in bird species richness when compared to earlier survey years. A single study on waterfowl described growth alterations in mallard ducklings raised on reclaimed wetlands, and a final two studies on waterbirds described increased egg mercury concentrations in birds nesting on lakes downstream of the oil sands, although the source of these increases could not be determined. These studies demonstrate a range of possible toxicological consequences that exposure to OSPW can cause. They also demonstrate that there is room for additional research, particularly regarding waterfowl contact with different types of process-affected water.

In this study, we assessed the health implications of waterfowl's contact with oil sands wastewater by exposing domestic ducks to recycled process-affected water. Our experimental procedure was designed to mimic the natural context in which birds primarily interact with the oil sands. Through evaluation of a range of biochemical, endocrinological, hematological, metal, and mineral analytes we found no evidence of detrimental physiological effects. Although we found some statistically significant differences, primarily as interacting effects with exposure or sex, all were within, or near available reference intervals for that species, which provides little evidence of toxicity. Although levels are likely not high enough to elicit toxic effects, significantly higher concentrations of vanadium in birds treated with OSPW may suggest it can be used as a

biomarker of exposure. Further research looking at the effects of OSPW on birds and other species may benefit from including a measure of vanadium in their research.

No other studies have investigated metals in bird blood in the context of the oil sands, although, mercury accumulation in eggs of colonial water bird nesting downstream of the oil sands was shown (Hebert et al., 2013, 2011). We were not able to measure mercury levels as part of this research project, but it would be a metal of interest for further studies.

This is only the second study examining the toxicological interactions between waterfowl and process-affected water, and the first to examine such a wide range of physiological analytes. This research has important considerations in the way migratory and resident birds are protected in the mineable oil sands region. Current practices aim to protect (deter) birds from all types of process-affected water, a strategy that is logistically challenging, as these ponds can be as large as 10 km^2 (St. Clair et al., 2012). One of the reasons we used recycled tailings pond water in our study was to be able to advance a sort of pond risk analysis. Current deterrent practices assume all ponds are equally hazardous and thus aim to deter birds from all of them equally. However, this is not the case as bitumen is much more harmful than OSPW, and bird protection should be applied accordingly to the risk individual tailings ponds pose. Our work supports that bird protection could be better focused on high-risk areas (e.g., those containing bitumen, fresh tailings, surrounding vegetation, etc.), with less effort allocated to 'low risk' water, such as recycled water ponds.

Appendix I: Linear Mixed Effect Models

Table I.1: Final model terms and validation for all biochemical, endocrinological, and hematological analytes in juvenile and adult birds. Residuals from the final model were examined for normality and heterogeneity, as well, the fitted values from the initial model were plotted against the final model, and the correlation was calculated.

Analyte	Age	Comments, Model Terms, Variance Weights (varIdent) or Correlation Structure	Correlation (%)
Weight	A	• Tarsal + Sex*Exposure + Sex*Treatment • Exposure*Sex	98.3
A:G	J	• Sex*Exposure + Exposure*Treatment • Sex	99.9
	A	• Sex + Exposure*Treatment • Treatment	98.6
Albumin	J	• Exposure + Sex*Treatment	99.8
	A	• Sex + Exposure*Treatment • Sex	97.5
Anion Gap	J	• Exposure*Treatment	99.7
	A	• Exposure*Treatment • Exposure	93.2
AST	J	• Sex*Exposure • Exposure	88.5
	A	• Sex*Exposure + Sex*Treatment • Exposure*Sex	67.9
Bicarbonate	J	• Exposure*Treatment	100
	A	• Sex*Exposure + Exposure*Treatment • corAR1(form = ~Exposure)	97.8
Bile Acid	J	• Exposure*Treatment • Exposure*Treatment	82.0
	A	• Sex*Exposure + Sex*Treatment • Exposure	55.1
Calcium	J	• Exposure + Sex*Treatment • Treatment	98.9
	A	• Exposure + Weight+ Sex*Treatment • Exposure*Sex	92.0
Chloride	J	• Sex*Treatment + Exposure*Treatment • Exposure*Treatment	96.4
	A	• Treatment + Weight+ Sex*Exposure • Exposure	98.0
Cholesterol	J	• Sex*Treatment + Exposure*Treatment • Exposure	94.6
	A	• Weight + Sex*Exposure + Exposure*Treatment • Exposure*Sex	99.0

Creatine Kinase	J	• Sex*Exposure • Exposure*Sex	78.4
	A	• Removed one outlier: Control female baseline = 1851 • Exposure + Weight+ Sex*Treatment • Exposure	91.3
GGT	J	• Residuals not normally distributed and evidence of heterogeneity. Tried removing outliers, GLMM but there was no improvement • Treatment • Exposure*Treatment	72.5
	A	• 14% 0's • Treatment + Sex*Exposure • Exposure*Sex	92.1
GLDH	J	• Residuals not normally distributed, evidence of heterogeneity Treatment + Sex*Exposure • Exposure*Treatment	80.9
	A	• 33% 0's • Residuals not normally distributed, evidence of heterogeneity • Sex*Exposure + Sex*Treatment • Exposure*Sex	84.9
Globulin	J	• Treatment + Sex*Exposure • Exposure	98.7
	A	• (Sex*Exposure*Treatment)^2 • Sex*Treatment	97.7
Glucose	J	• Sex*Exposure • Exposure	72.8
	A	• Sex*Exposure + Exposure*Treatment • Exposure*Sex	97.8
Phosphorus	J	• Exposure*Treatment	100
	A	• Treatment + Sex*Exposure • Exposure*Sex	97.9
Potassium	J	• Exposure*Treatment	100
	A	• Sex + Weight + Exposure*Treatment • Exposure	38.9
Sodium	J	• Sex + Exposure + Treatment • Exposure*Treatment	89.2
	A	• Weight + (Sex*Exposure*Treatment)^2 • Exposure*Treatment	81.8
Total Protein	J	• Exposure + Sex*Treatment • Exposure	97.5
	A	• Sex*Treatment + Exposure*Treatment • Exposure*Sex	81.8

Uric Acid	J	• Exposure*Treatment • Exposure	98.6
	A	• Removed one outlier: Treatment male baseline =1019 • Sex + Weight + Exposure*Treatment • Exposure*Sex	85.7
Corticosterone	J	• Sex + Exposure*Treatment • Exposure	84.4
	A	• Sex*Treatment + Exposure*Treatment • varPower (form= ~fitted (.))	93.8
T3	J	• Treatment • Exposure	84.9
	A	• Sex + Weight + Exposure*Treatment • Exposure*Sex	95.9
T4	J	• Exposure + Sex*Treatment • Sex	97.1
	A	• Sex*Treatment • Sex*Treatment	85.2
Thyroid	J	• Exposure + Sex*Treatment • Exposure	95.9
	A	• Sex + Exposure • Exposure*Sex	58.4
Basophils	A	• Sex + Exposure*Treatment • Sex	93.9
Eosinophils		• 74% 0's • Sex*Treatment + Exposure*Treatment, family = binomial	76.5*
Heterophils		• Sex + Exposure • Sex	91.2
Lymphocytes		• Exposure + Sex*Treatment • Exposure	91.8
Monocytes		• Weight + Sex*Exposure + Exposure*Treatment • varPower (form= ~fitted (.))	93.9
PCV		• Sex*Treatment + Exposure*Treatment	99.3
WBC		• Sex + Exposure • Sex	89.0

Note: *Model fit for eosinophils was evaluated by plotting residuals and the area under the ROC.

Appendix II: Additional Figures

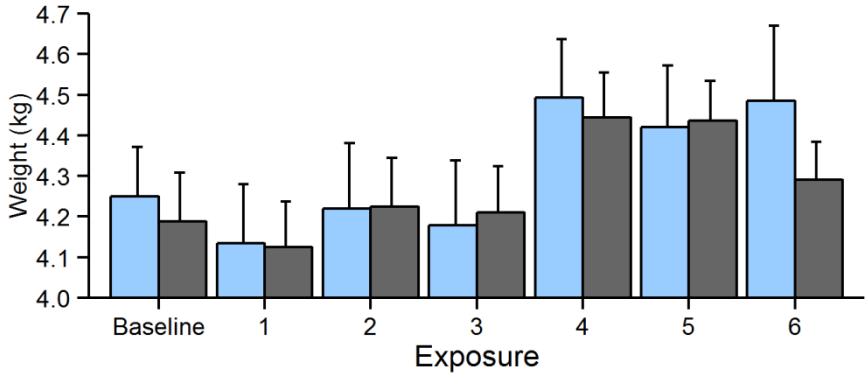

Figure II.1: Mean (± SE) weight in adult ducks across the second trial exposed to control and OSPW (blue and grey bars, respectively).

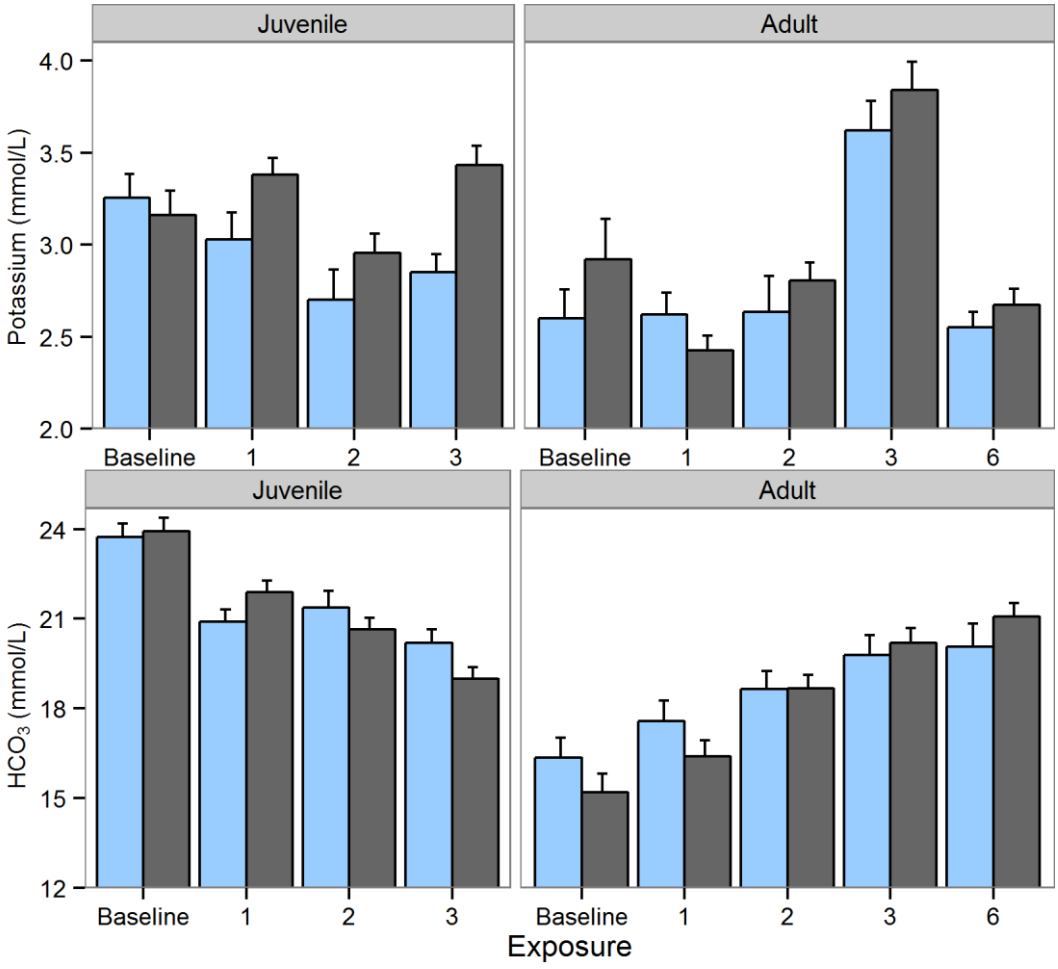

Figure II.2: Mean (± SE) levels of the electrolytes potassium and bicarbonate in juvenile and adult birds exposed to control and OSPW (blue and grey bars respectively).

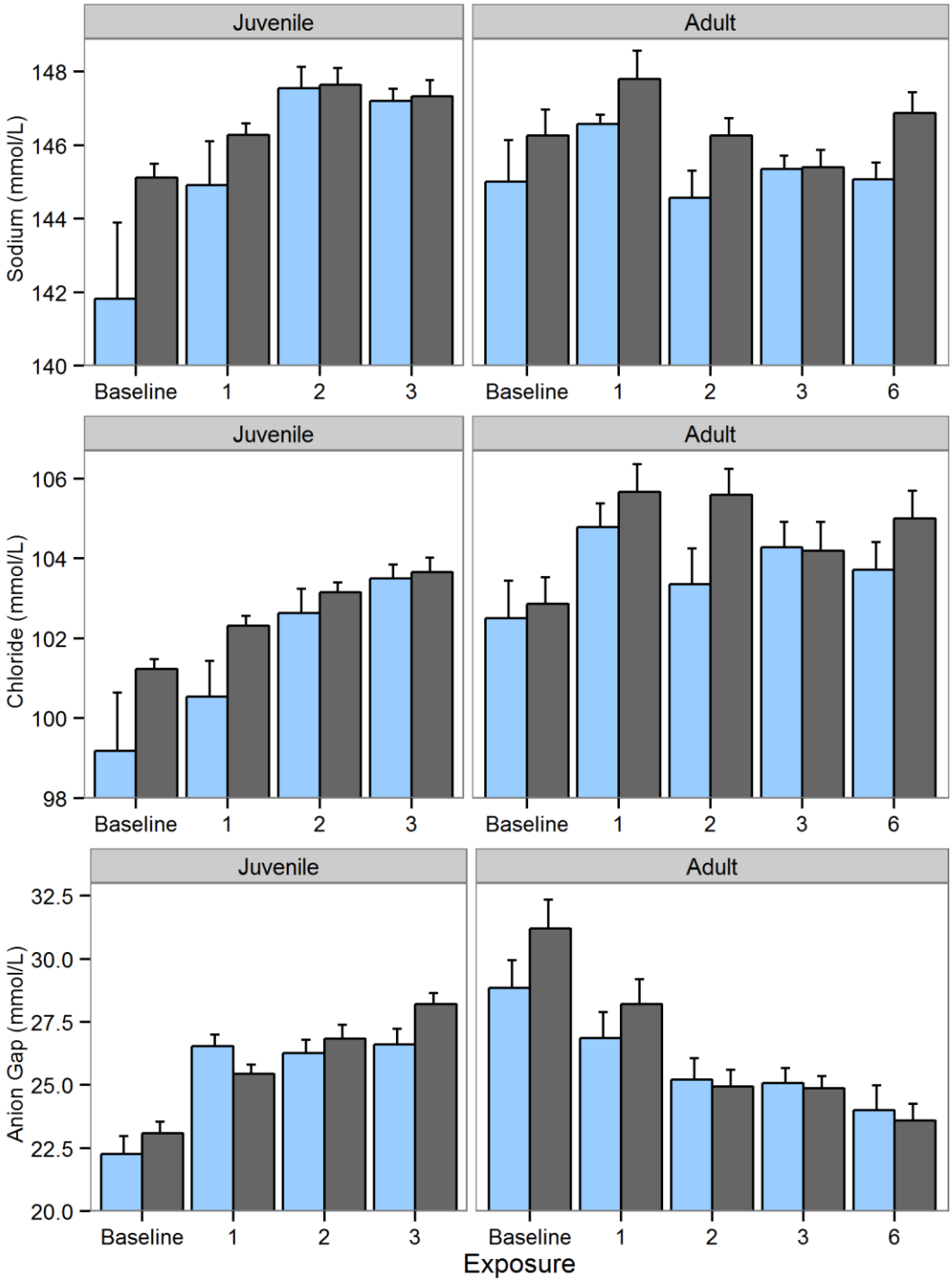

Figure II.3: Mean (± SE) levels of the electrolytes sodium, chloride, and the anion gap in juvenile and adult birds exposed to control and OSPW (blue and grey bars respectively).

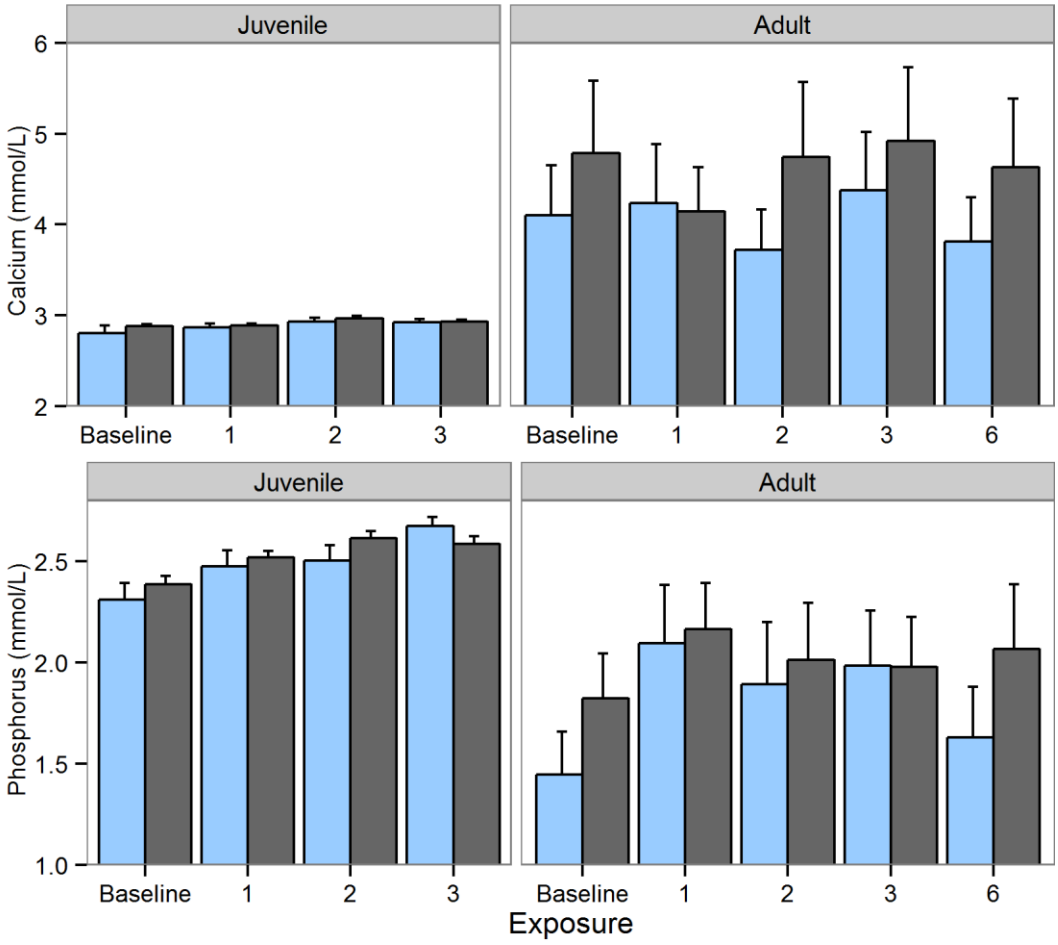

Figure II.4: Mean (± SE) levels of metabolites calcium and phosphorus in juvenile and adult birds exposed to control and OSPW (blue and grey bars respectively).

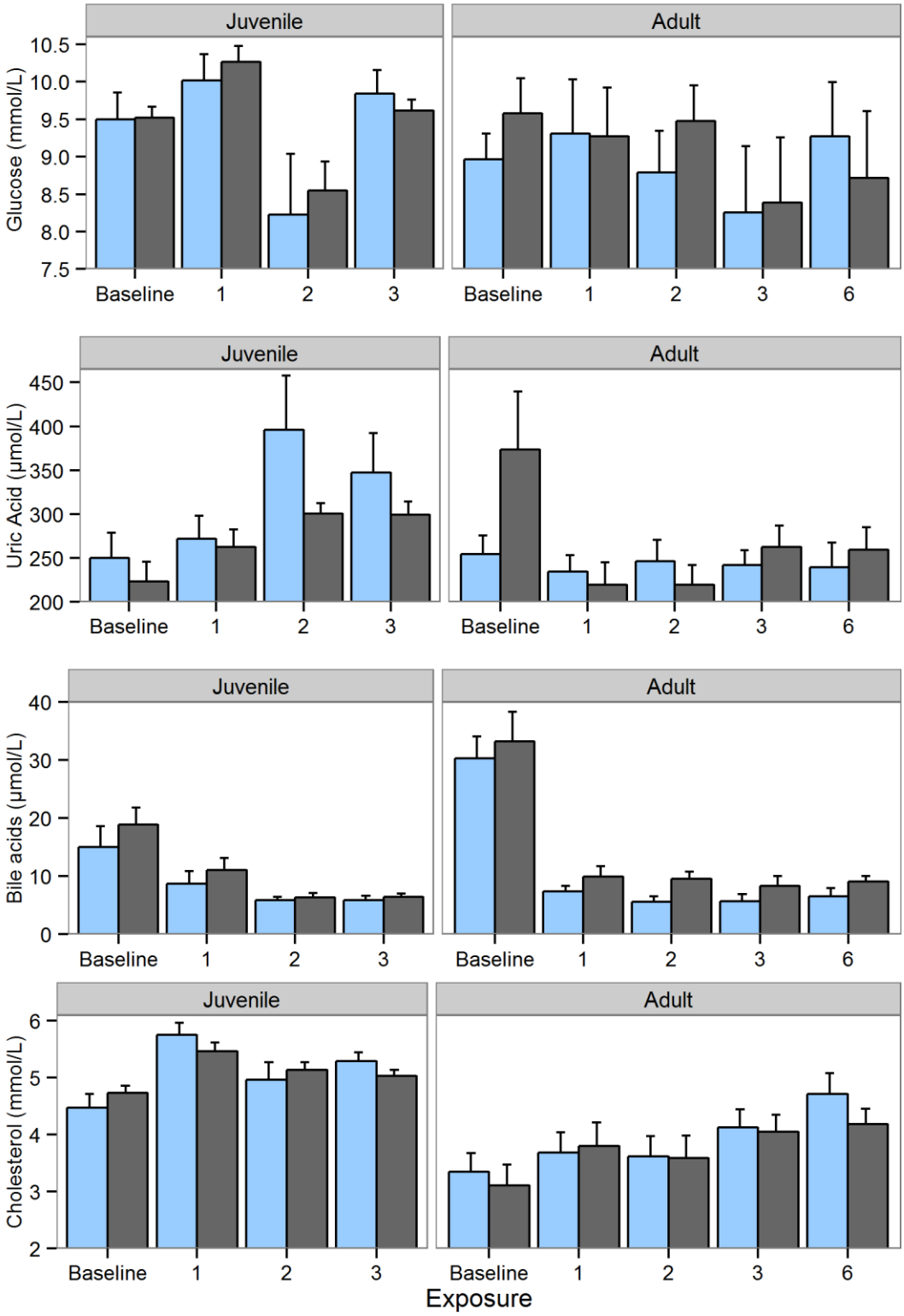

Figure II.5: Mean (± SE) levels of the metabolites glucose, uric acid, bile acids and cholesterol in juvenile and adult birds exposed to control and OSPW (blue and grey bars respectively).

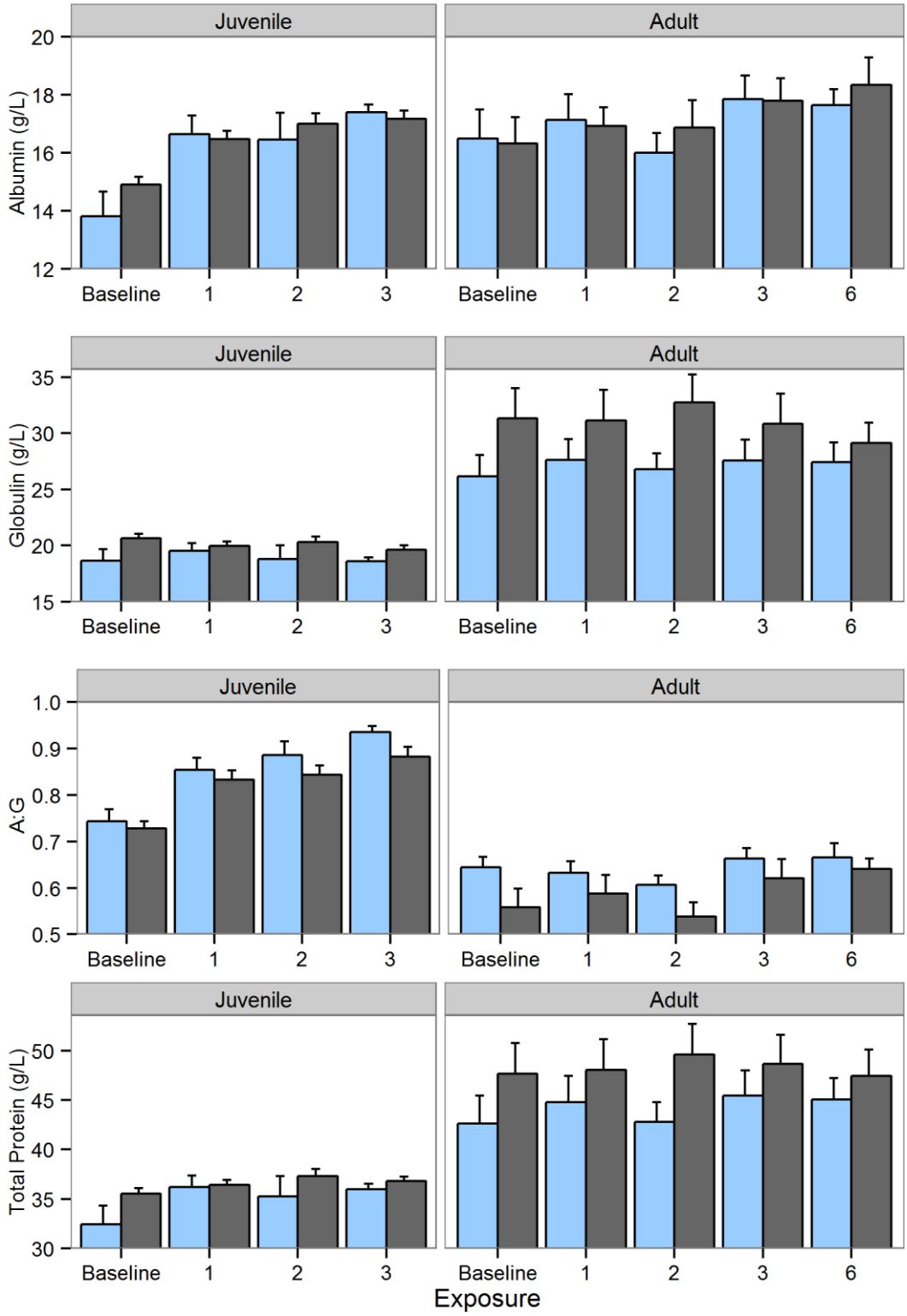

Figure II.6: Mean (± SE) levels of albumin, globulin, the A:G ratio, and total proteins in juvenile and adult birds exposed to control and OSPW (blue and grey bars respectively).

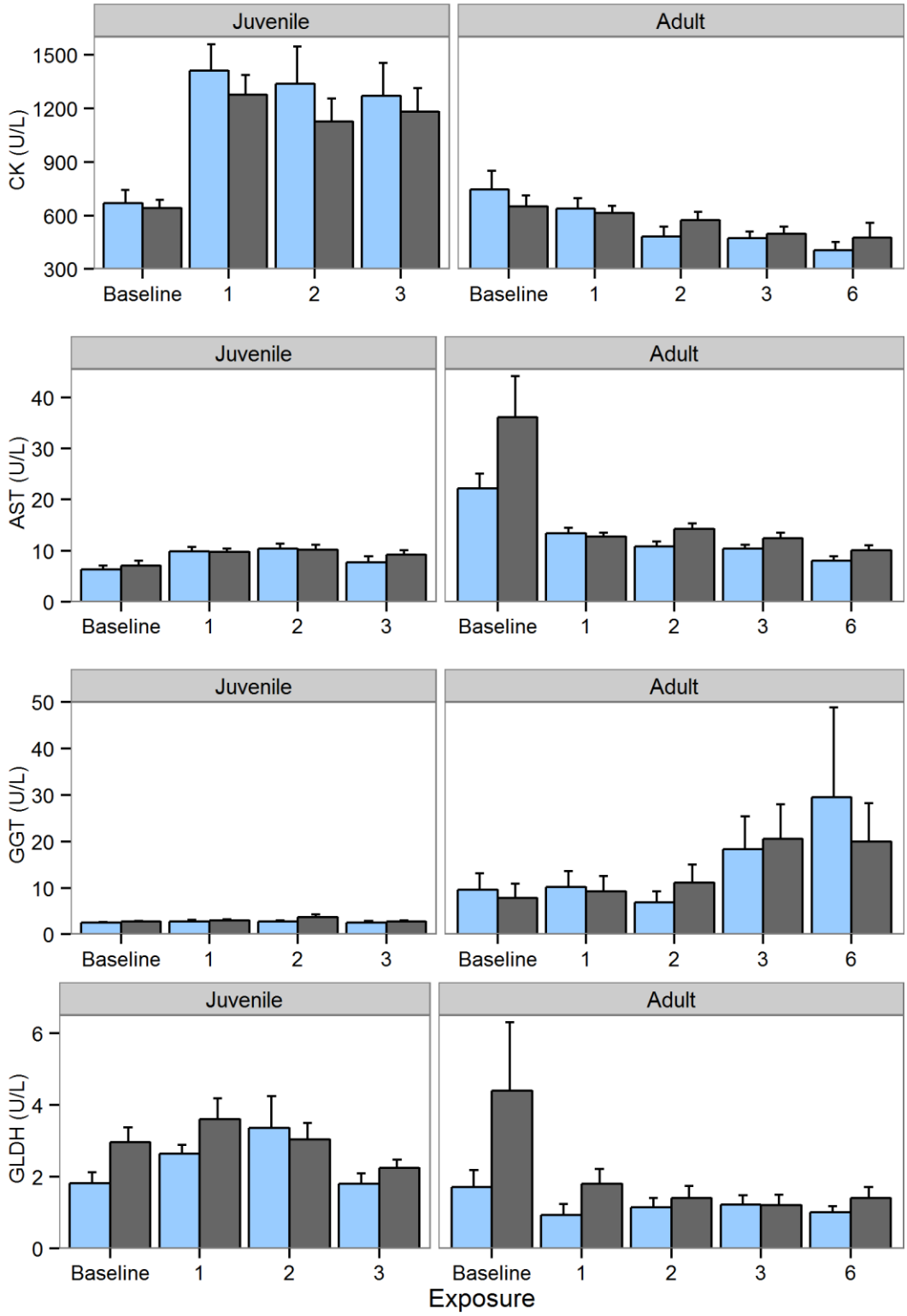

Figure II.7: Mean (± SE) levels of the enzymes CK, AST, GGT and GLDH in juvenile and adult birds exposed to control and OSPW (blue and grey bars respectively).

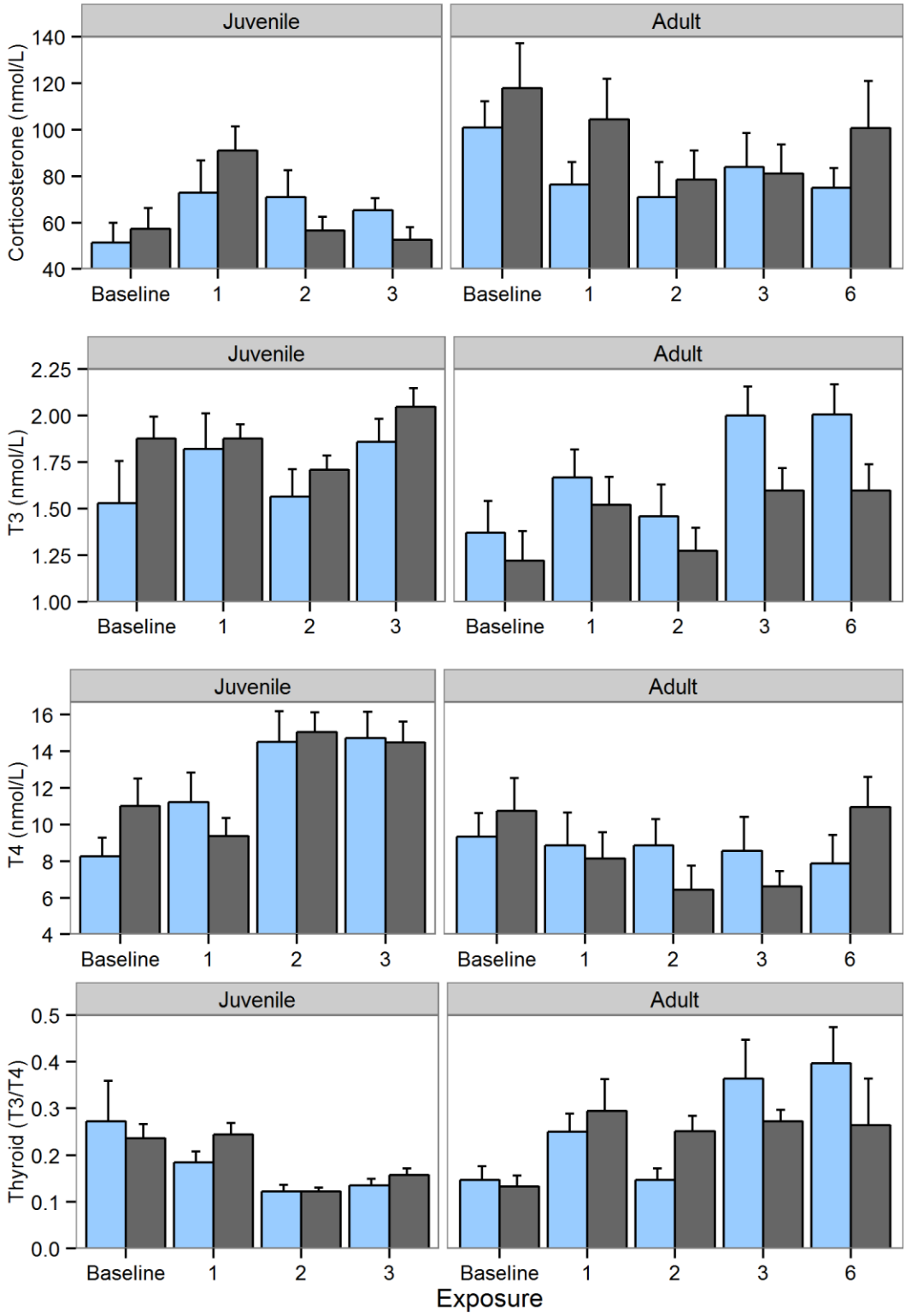

Figure II.8: Mean (± SE) levels of the hormones corticosterone, T3, T4 and thyroid hormone ratio (T3/T4) in juvenile and adult birds exposed to control and OSPW (blue and grey bars respectively).

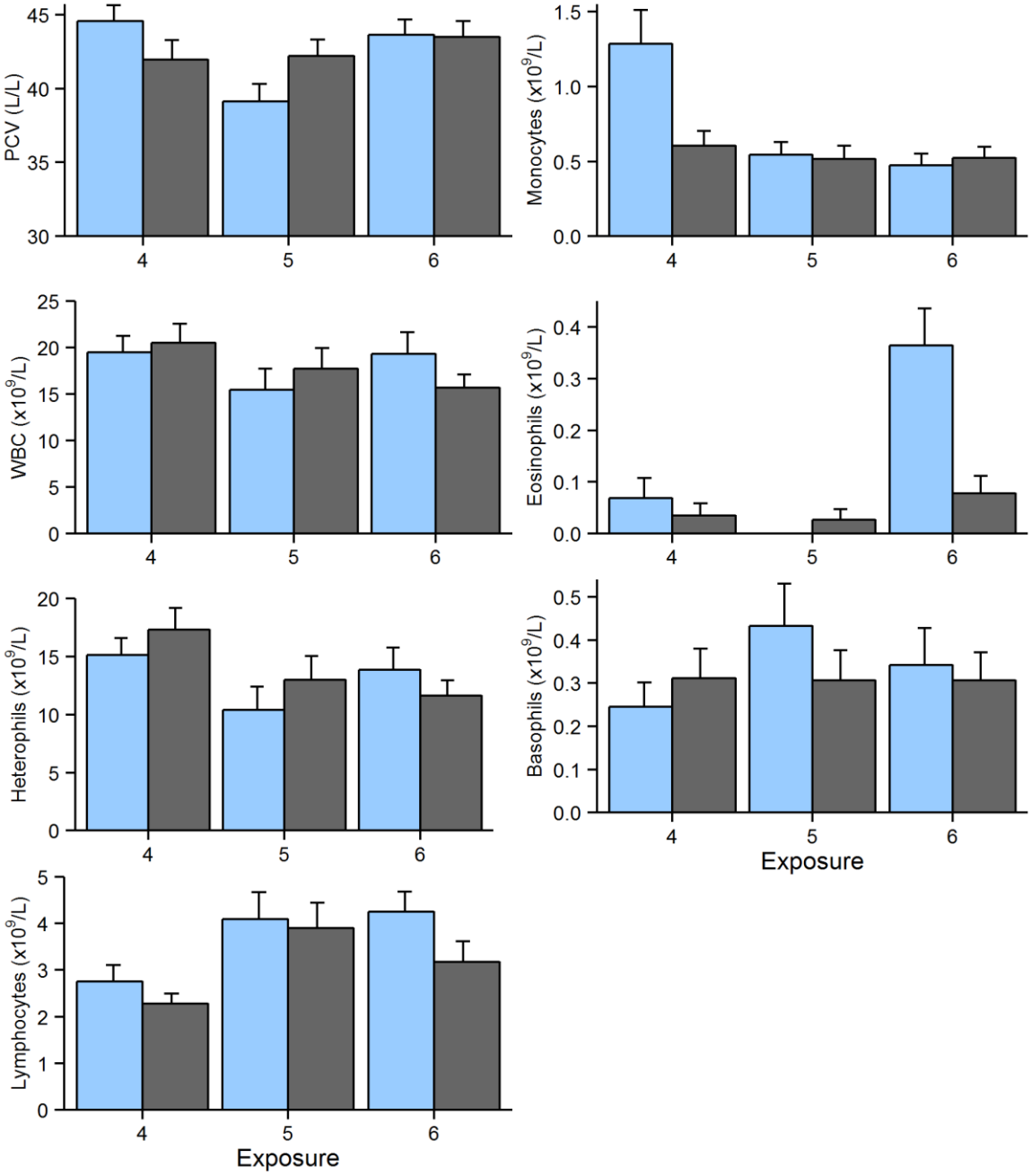

Figure II.9: Mean (± SE) levels of measured hematology analytes in adult birds exposed to control and OSPW (blue and grey bars respectively).

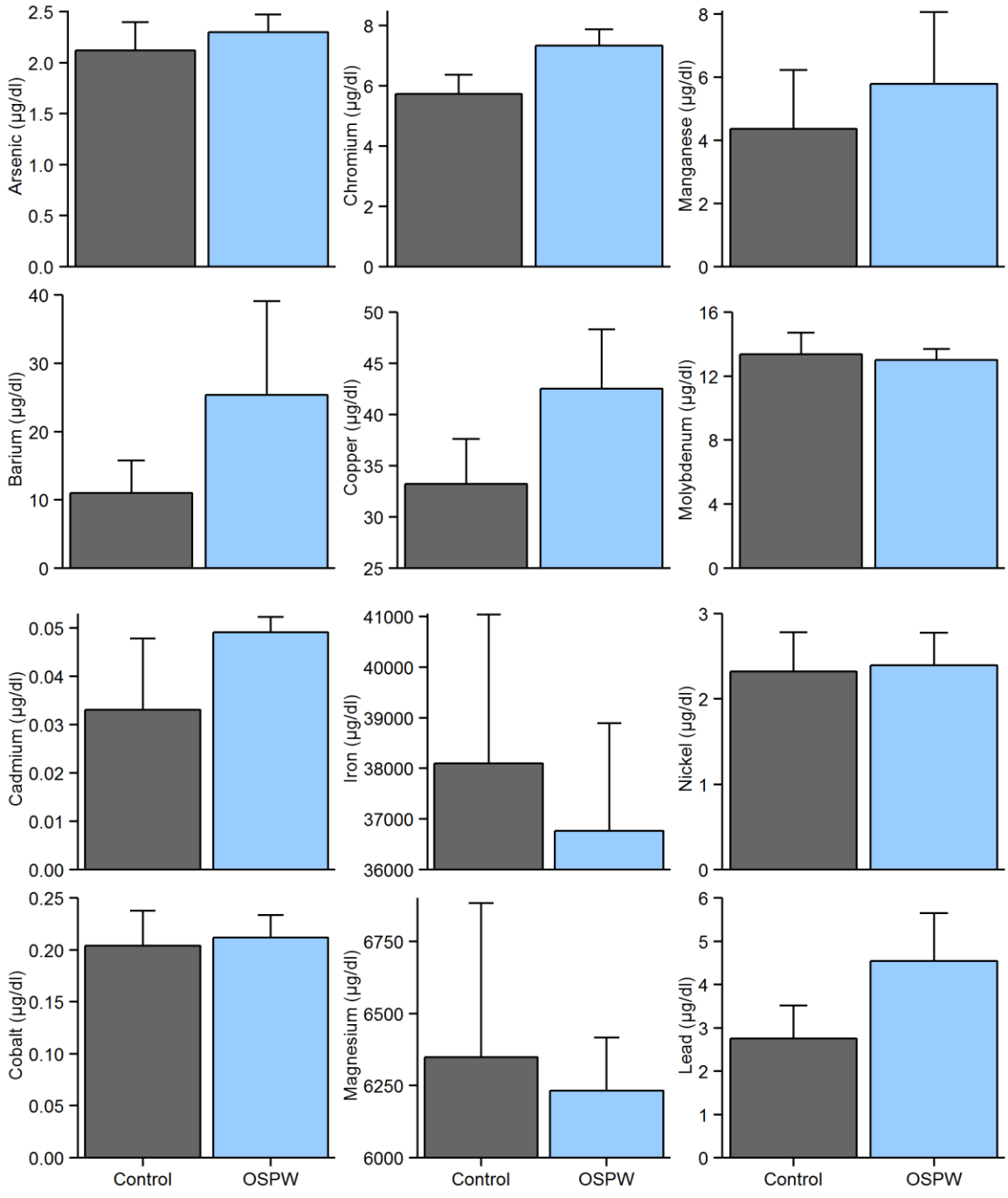

Figure II.10: Mean (± SE) blood metal concentrations from the final exposure in adults birds exposed to control and OSPW (blue and grey bars respectively).

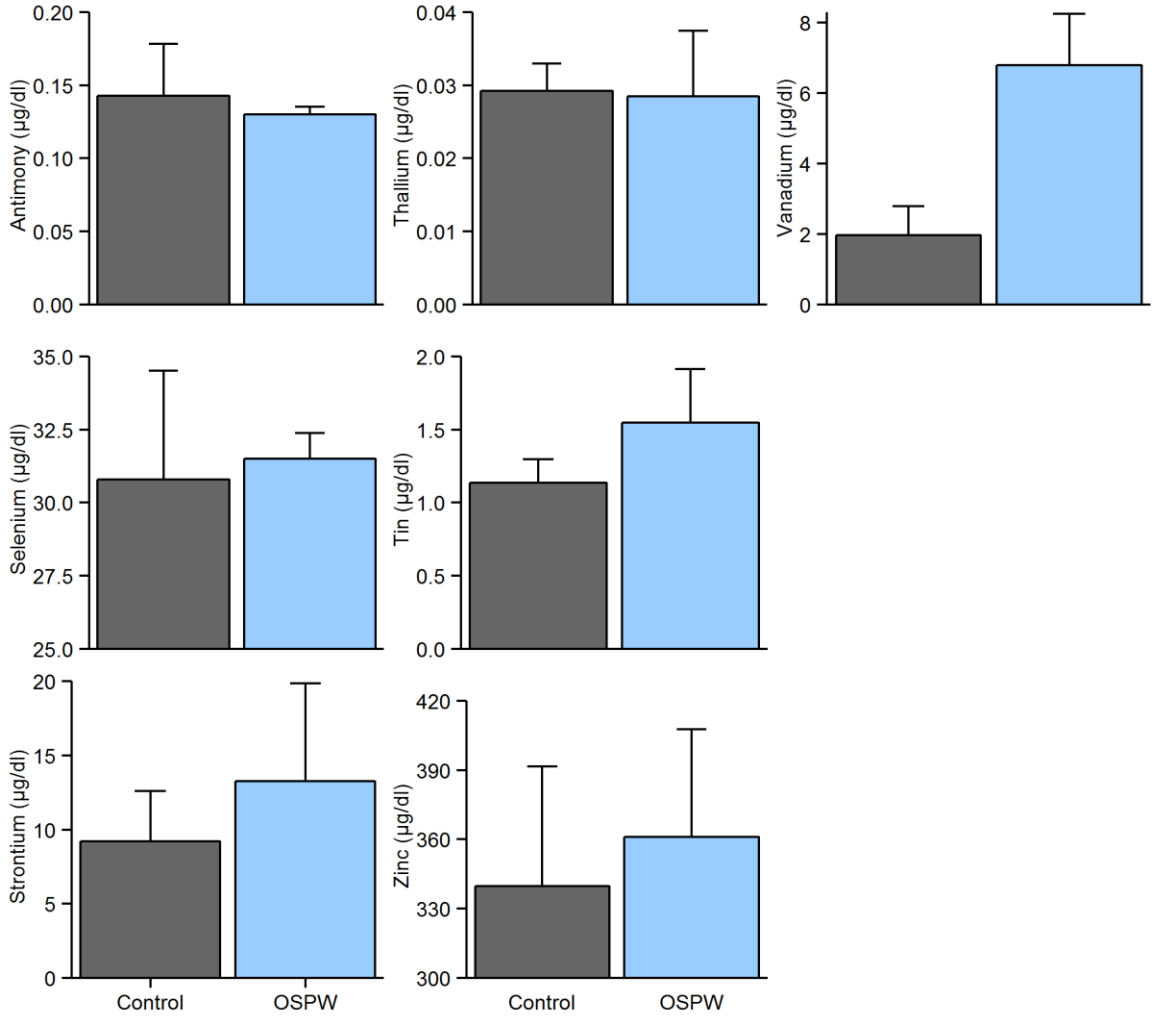

Figure II.11: Mean (± SE) blood metal concentrations from the final exposure in adults birds exposed to control and OSPW (blue and grey bars respectively).

Appendix III: Can Exposure To Petrochemicals Be Non-Invasively Detected After Contact?

One of the initial objectives of this thesis was to determine whether exposure to oil sands process-affected water could be non-invasively detected by use of adsorbent material swabs. Specifically, we wanted to measure deposits of PAHs or other bitumen related compounds on the feathers of birds after their short-term exposures to OSPW. The development of a non-invasive method to detect exposure to oil sands compounds would pave the way for field assessment of free-living ducks on tailings ponds or other neighboring areas. This has potential applications to hunters who are concerned over the contamination of their food source (the birds), as well as government biologists responsible for monitoring the impact of the oil sands on bird populations. Although this project was never completed, many avenues were explored. I present here a very brief summary of the directions we investigated, in the hope that future research will expand upon this.

Methods for hydrocarbon detection have primarily been developed for water and sediment samples. These types of tests are fairly standardized, and can be requested or ordered from major analytical labs. Methods might include gas chromatography and mass spectroscopy, which are time consuming and expensive, and require qualified operators of laboratory equipment (Fritcher et al., 2002).

Several methods for detecting oil pollution specific to birds have been described in the literature. Birds can be visually inspected for oiling, although this can be difficult in birds with darker plumage. Post mortem examinations can also be used, for example, Troisi et al. (2006) quantified polycyclic aromatic hydrocarbons and metabolite tissue burdens in oil contaminated common guillemots using gas chromatography-mass spectroscopy. Custer et al. (1994) used a flow cytometry method to determine cytogenetic damage in black-crowned night herons living on sites contaminated with petroleum. Detection of hydrocarbons in plasma samples is another option; Troisi and Borjesson (2005) modified a PAH immunoassay to detect concentrations in plasma samples.

Immunoassays have been increasingly used to detect PAHs in sediment samples, and have been applied to detect oil contamination on mink fur (Mazet et al., 1997). Applications to birds, however, are limited. Fritcher et al. (2002) tested the RaPID Assay and EnviroGard^{TM} for their ability to detect oil contamination on bird feathers. They measured sensitivity and specificity, and determined that both were viable methods due to their speed and low cost. EnviroGard was the preferred method, also due to the speed of results and its ease of use. However, at the time we were investigating these options this assay was no longer available.

As no standardized method currently exists, the objective of this project was to develop such a method. We wanted to use this technique to determine if we could detect bitumen in the cloths that had been used to dry ducks that had bathed in recycled process-affected water. The exposure methods are the same as those described in chapter 3. Briefly, ducks were exposed to either tap water or OSPW dependent upon their experimental allocation. Each duck was exposed for 6–8 hours on three occasions as juveniles (n = 108), and 6 occasions as adults (n = 174). As ducks were removed from water bins, their underbellies were swabbed with an oleophilic material. The wipes were immediately placed into mason jars and sealed, and labelled with duck ID, experimental group, exposure, and sampling data.

We discussed this project with several experts across the engineering, chemistry, and medical sciences faculties (Grey, 2012 pers. comm.; Lucy, 2013 pers. comm.; Martin, 2013 pers. comm.) The first option we considered was to use toluene extraction followed by multipoint UV-visible spectroscopy method (Grey, 2012 pers. comm.). This option would have involved developing a standard, or calibration curve, followed by comparing the absorbance of the cloths to this curve. A second avenue we considered was to use an immunoassay (Lucy, 2013 pers. comm.). However, this method is costly, requires specialized equipment, and is not necessarily accessible (the suggested kit is no longer available). Another suggestion was to equilibrate samples in water, and analyze this water for common compounds (PAHs, NAs etc.) by an analytical lab such as ALS Environmental (Edmonton, AB, Canada; Lucy, 2013 pers. comm.). The issue we foresaw with this method was that concentrations of our variables of interest in the recycled water were low, and after equilibrating the towels in the water, followed by quantification, it is

likely that concentrations would be below the detection limit of the analysis (i.e. not sensitive enough). Additionally, this method would not be cost effective. Another avenue we considered was also based on the water analysis provided by ALS. We determined that measurement of the hydrocarbon fraction, F3 would likely be the best indicator of exposure to OSPW (Martin, 2013 pers. comm.). ALS could perform this analysis by first extracting the wipes with hexane, however again this is not a cost effective or timely option.

Although we were not able to test any of these methods, the concept remains an interesting prospect for future work. The public is concerned over birds landing and dying in tailings ponds, yet few consider the implications of those that land but do not die. These birds may become contaminated with hydrocarbons and fly away likely entering various food chains, including ours. The ability to detect whether birds being consumed by humans have been contaminated with oil sands pollutants is therefore important for our health and safety.

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