Health Assessment of Tree Swallows (*Tachycineta bicolor*) Nesting on the Athabasca Oil Sands, Alberta

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ABSTRACT

Oil sands mining companies in Alberta, Canada, are planning to create wetlands for the bioremediation of mining waste materials as part of a reclamation strategy. To assess feasibility, experimental wetlands mimicking proposed reclamation scenarios were constructed on mining leases. This research assessed the health of tree swallows (*Tachycineta bicolor*) nesting on these sites where they were naturally exposed to a mixture of chemicals including unrecovered bitumen, naphthenic acids (NAs) and polycyclic aromatic hydrocarbons (PAHs). Endpoints reflecting health were compared among three experimental wetlands and one reference site. In order to specifically investigate toxicity of NAs to birds, an experimental exposure to NAs was also conducted on a subset of nestlings on the reference site.

In 2003 and 2004, approximately 50 breeding pairs (total, per year) nesting on the following sites were monitored: Suncor's "Consolidated Tailings" and "Natural Wetlands"; Syncrude's "Demo Pond" and "Poplar Creek" reference site. In 2003, reproductive success was very low on OSPM-sites compared to the reference site, but was relatively unaffected in 2004. Compromised reproductive performance in 2003 was linked to harsh weather, during which mortality rates of nestlings reached 100% on the site with the highest levels of PAHs and NAs, while they did not surpass 50% on the reference site. In 2004, mortality rates were low but nestlings from OSPM-sites weighed less and showed greater hepatic detoxification efforts (etoxyresorufin-o-deethylase activity) than those on the reference site. Furthermore, nestlings on OSPM-sites exhibited higher levels of thyroid hormones and suffered parasitic burdens (*Protocalliphora spp.*) approximately twice that of those on the reference site. Several of these findings may be associated with low post-fledging survival, suggesting that wet landscape reclamation strategy is not optimal for avian species and may require improvement.

As part of a separate study investigating toxicity of naphthenic acids, twenty nestlings from the reference site were randomly selected for an experimental exposure.

Nestlings received 0.1 ml/day of NAs (15g/L) orally from day 7 to day 13 of age while being reared normally by their free-ranging parents. Nestling growth, hematocrit, blood biochemistry, organ weights and etoxyresorufin-o-deethylase activity (EROD) activity appeared unaffected by naphthenic acids. No toxic changes were detected on histopathological evaluation of major organs. These findings suggest that for nestlings reared on oil sands reclaimed sites, exposure to other chemicals such as polycyclic aromatic hydrocarbons is a greater concern than exposure to NAs. However, this study did not investigate the chronic or reproductive toxicity of naphthenic acids. More research still needs to be conducted as a part of an assessment of the sustainability of wet landscape reclamation because a previous study found that chronic exposure to NAs severely compromised reproduction in mammals.

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LIST OF ABREVIATIONS

AOSTRA: Alberta Oil Sands Technology and Research Authority

C: Celcius

CEATAG: CONRAD Environmental Aquatics Technical Advisory Group

CONRAD: Canadian Oil Sands Network for Research and Development

CT: consolidated tailings

d: day

D: day

DP: Demo Pond

EROD: etoxyresorufin-o-deethylase

FTFC: Fine tailings fundamental consortium

FTIR: Fourier transform infrared

g: gram

GC-MS: gas chromatography-electron impact spectrometry

i.m.: intramuscular

i.p.: intraperitoneal

LC_{50}: median lethal concentration

LC_{50}: median lethal dose

MFT: mature fine tailings

mg: milligram

ng: nanogram

µg: microgram

MLSB: Mildred Lake settling basin

MW: molecular weight

NAs: naphthenic acids

NW: Natural Wetlands

OS: oil sands

OSPM: oil sands process-affected materials

OSPW: oil sands process-affected water

PAHs: polycyclic aromatic hydrocarbons

PC: Polar Creek

RIA: radio-immuno assay

INTRODUCTION

Oil sands mining companies in Alberta, Canada, are planning reclamation strategies in which wetlands will be used for the bioremediation of water and sediments affected by the oil sand extraction process. To assess the sustainability and feasibility of these reclamation plans, a series of experimental wetlands and test ponds mimicking future reclamation scenarios were constructed and filled with mine tailings on the two major companies' leases. The first part of this research consists of an assessment of the health of tree swallows (*Tachycineta bicolor*) nesting on those experimental wetlands, where they were exposed to a mixture of chemicals from Oil Sands Process Materials (OSPM) which include naphthenic acids (NAs) and polycyclic aromatic hydrocarbons (PAHs). The second part of this project was aimed at specifically investigating the toxicity of naphthenic acids (NAs) to a native avian species. Naphthenic acids are one of the main contaminants of concern for the oil sands industry, and yet no data on avian toxicity are available. This experiment was meant to address this lack of information.

The main chapters of this thesis were written as manuscripts destined for publication in scientific journals. A literature review (chapter 1) precedes the main body of the thesis. It includes a description of the oil sands mining industry and of the environmental issues associated with the management of mine tailings, and summarizes available data on the chemistry and toxicology of naphthenic acids. It also includes a description of the study sites and of the study animal (the tree swallow), as well as a list of avian species living in the research area. The first three manuscripts (chapters 2, 3, 4) encompass the different aspects of the health assessment conducted on OSPM-sites, which were all measured on the same set of nests and nestlings. Chapter 2 focuses on population dynamics and includes the evaluation of traditional avian ecotoxicological endpoints such as reproductive success, nestling growth and nestling hepatic detoxification activity (ethoxyresorufin-*o*-deethylase, EROD). It also describes a widespread nestling die-off that occurred in 2003 and contains meteorological data for the summers of 2003 and 2004. Chapter 3 investigates parasitism of nestlings with larvae of the blowfly *Protocalliphora sp.*, while chapter 4 evaluates the thyroid function of nestlings. The last manuscript (chapter 5) describes the experimental exposure of nestlings to NAs. This was conducted on a subset of nests on the reference site in 2004 and should be viewed as a separate study. The general discussion (chapter 6) highlights the major findings of this research and their implications regarding wet landscape reclamation.

Notice: Background information on oil sands mining is presented in the introduction section of each chapter and varies according to the focus of the manuscript, but some repetition is to be expected. For methodological material that is identical in all chapters regardless of the manuscript topic (example: description of the study sites), the reader is referred to the section where these particular methods were initially described.

RESEARCH OBJECTIVES

1. To evaluate the sustainability of "wet landscape" reclamation for upper trophic level avian species using tree swallows as a model.

-To characterize adverse effects on health and reproduction of tree swallows nesting on experimental wetlands containing waste materials from the oil sands extraction process.

2. To investigate the toxicity of naphthenic acids (NAs) to birds using tree swallows as a surrogate species.

-To characterize adverse effects on health following experimental sub-chronic exposure of nestlings tree swallows to NAs

3. To assess whether NAs present in oil sands waste materials pose threat to insectivorous passerines that colonized reclaimed areas.

-To compare the results from the experimental exposure to NAs with those from the health assessment of tree swallows on oil sands reclaimed sites.

CHAPTER 1

Literature Review

1.1. THE OIL SANDS INDUSTRY IN CANADA

1.1.1. The Oil Sands of Northeastern Alberta

Oil deposits impregnated with sand occur in more than 70 countries, the largest deposits being in the Orinoco region of Venezuela and in Alberta, Canada. Smaller ones are found in the USA (Utah). The three oil sands deposits of Alberta (Athabasca, Peace River and Cold Lake) cover over 140,000 km^2. The largest one, Athabasca, covers approximately twice the size of Lake Ontario (about 42,000 km^2) and is the largest oil deposit in the world (Syncrude Canada Ltd., 2005). With the worldwide decline of conventional fossil fuel, development of the vast economic potential of the oil sands reserves has become a priority. According to the Alberta Department of Energy (2005), bitumen extracted from the oil sands currently represents 54% of Alberta's total oil production, and about one-third of all the oil produced in Canada. Within five years, production from the oil sands is expected to reach 50% of Canada's total crude oil output, and 10% of North American production. Suncor Energy Inc. and Syncrude Canada Ltd. are the two largest mining companies operating on the oil sands. Syncrude is the world's largest single source oil producer and its production supplies 13% of Canada's energy requirements (Syncrude Canada Ltd., 2005).

1.1.2 Oil Sand Mining

Oil sands, previously known as tar sands, are sand deposits impregnated with bitumen, a category of heavy crude oil which is highly viscous, black and sticky.

Average ores typically contain 9-12% bitumen by weight (Strausz and Lown, 2003). The remainder is composed of 75-84% clay and quartz sands, and around 4-6% water (AOSTRA, 1989). Because bitumen has the viscous consistency of molasses, it cannot be recovered by conventional methods such as well drilling.

1.1.2.1 Surface Mining

Open-pit mining can be used to recover bitumen from deposits close to the surface. Current techniques allow access to oil sands situated as deep as 75m, but some deposits in Alberta are buried under as much as 90m of overburden. Only 7% of the reserves from the Athabasca region can be harvested using surface mining (Alberta Department of Energy, 2005). Open-pit mining involves the stripping of all vegetation and topsoil, as well as landscaping to channel natural drainage, resulting in serious alteration to wildlife habitat. The overburden is then stockpiled so it can be used for dyke construction on the mining sites and future terrestrial reclamation. About two tons of oil sands have to be dug up, moved and processed to produce one 159-litre barrel of crude oil (Bott, 2005). The cumulative land disturbance associated with surface mining to date is over 150 km^2, and some estimates suggest that by the year 2023 the affected area may be as much as 10 times larger, potentially exceeding 1,406 square kilometers (OSERN, 2004).

1.1.2.2 In Situ-Mining

For reserves that lay too deep to be recovered by surface mining, in-situ mining techniques are being developed. These involve the injection of high-pressure steam through vertical wells into the deposits. Once heated, bitumen has lower viscosity and can be channeled toward extraction wells, where it is recovered following traditional oil drilling techniques. In-situ techniques are mainly used in the Cold Lake and Peace River deposits (Alberta Department of Energy, 2005).

1.1.3 Extraction of Bitumen from Oil sands

Separation of the heavy crude oil from sand is accomplished by the Clark Hot Water Extraction Process. A detailed review of this process can be found in the publications of the Fine Tailings Fundamental Consortium (FTFC, 1995). Briefly, the oil sands are crushed, then mixed with hot water (80ºC) and sodium hydroxide (NaOH) in rotary breakers (tumblers) to form a slurry. Sodium hydroxide (caustic soda) promotes the separation of bitumen from sand particles through the liberation of natural surfactants present in oil sands (FTFC, 1995). Air trapped in the slurry creates froth where bitumen coats air bubbles. The froth floats and can easily be skimmed off to recover bitumen, while sand fall to the bottom of the separation vessels (Bott, 2005). Once the bitumen has been extracted, residual sand, clay, unrecovered bitumen and water are diverted to large settling basins (called tailings ponds) on the mining sites (Mikula et al., 1996).

1.2. ENVIRONMENTAL DISTURBANCE FROM OIL SANDS MINING

1.2.1 Mine Tailings

The processing of one cubic metre of oil sands generates four cubic meters of tailings, which are accumulating at the rate of ~10^5 m^3/day (Madill et al., 2001). The main tailings pond at Syncrude, Mildred Lake Settling Basin (MLSB), presently covers 20-25 km^2 (OSERN, 2005). Since there is currently no release of the mining effluents off the leased areas, as much as 1 billion m^3 of oil sands tailings will require detoxification and reclamation when mines are decommissioned (FTFC, 1995). Integration of such large volumes of contaminated water into the environment represents a major challenge for the industry.

Oil sands tailings consist of the ore body (sand, silt and clays) and of water added during extraction. They also contain unrecovered bitumen. In settling basins, coarse mineral particles quickly segregate to the bottom. The top layer of tailings ponds (about 3m deep) is mostly water and is recycled back to the extraction operations. The remaining sludge is called “fine tailings”. Fine tailings have a very slow settling rate because of their salinity, which causes clay particles to repel each other and keeps them suspended (FTFC 1995). Over approximately five years, the fine tailings slowly become more dense, reaching a stable semi-fluid state called “mature fine tails” (MFT). Syncrude’s MFT contain about 85% water, 13% clay and 2% bitumen (OSERN 2005). Because MFT have such high water content, they retain fluid characteristics and must be stored behind dykes with little possibility of being used as a substrate for vegetation. It may take decades or centuries before they become solid enough to support the weight of vehicles (FTFC 1995). Techniques for increasing the settling rates of tailings are under development and involve the addition of coagulants such as gypsum to form consolidated or composite tailings (Golder Associates Ltd. 1998).

The acronym “Oil Sands Process-Affected Materials” (OSPM) is now used by the members of the industry to replace the term “tailings”. Technically, Oil Sands Process-Affected Materials are composed of mature fine tails (MFT) and of Oil Sands Process-Affected Water (OSPW), which is the pore water released from the tailings as they become more compact. This pore water moves up to the surface of the settling ponds, in a process called “dewatering of the MFT”.

1.2.2 Wet Landscape Reclamation

Wet landscape reclamation involves transferring the tailings from their settling ponds into excavated areas such as mined-out pits and constructed wetlands then capping them with a layer of clean water to ensure the isolation of the underlaying fine tails. Because tailings have high density and low permeability, minimal physical mixing of the distinct layers is expected (Lawrence et al., 1991). The objective of wet landscape reclamation is the creation of artificial water bodies, with the appearance and productivity of naturally occurring wetlands and lakes in the region (Gulley and MacKinnon, 1993). Reclaimed sites are expected to eventually evolve into selfsustaining ecosystems recolonized by native flora and fauna. However, because the gradual consolidation of the fine tail zone causes the release of contaminated pore water into the water cap, toxic chemicals may still affect biota inhabiting the reclaimed areas. To study the feasibility, effectiveness and viability of this reclamation option, Suncor and Syncrude have both constructed on their leases a number of small-scale experimental wetlands and test ponds, which they have partly filled with tailings. Multiple research projects from several universities have been and are currently being conducted on these experimental sites, which will be referred to as "OSPM-wetlands".

1.2.3 Chemical Characteristics of Oil Sands Process-Materials (OSPM)

The main contaminants in OSPM are parent and alkylated polycyclic aromatic hydrocarbons (PAHs), dibenzothiophenes (heterocyclic PAHs containing sulphur), metals and naphthenic acids (Parrott et al., 1996). Available information about the chemistry and toxicology of naphthenic acids is summarized in Section 1.3 of this thesis. Polycyclic aromatic hydrocarbons found in OSPM mainly originate from the weathering of unrecovered bitumen. The PAHs from petroleum sources, unlike those from pyrogenic sources (combustion), show greater abundance of alkylated homologues rather than parent (non-alkylated) compounds (Voudrias and Smith, 1986). Because most of the research on PAHs has focused on parent compounds, little is known about the bioaccumulation potential or relative toxicity of alkylated homologues. Thus, it is difficult to determine whether or not the levels of PAHs in reclaimed wetlands are of concern. Concentrations of total alkylated polycyclic aromatic hydrocarbons in were 6-13 time higher in sediments of OSPM-wetlands than in those of unaffected areas in 1998 (Smits et al., 2000).

Elevated salinity, dominated by sodium, sulfate and chloride ions, is characteristic of OSPW. Salinity in water bodies created during wet landscape reclamation will range from 3-5 g/L (Leung et al., 2003). As a comparison, sea water contains approximately 35g/L of salt. Many fresh-water plants and invertebrates are very salt-intolerant, and these concentrations are sufficient to cause substantial shifts in aquatic communities (Hart et al., 1990). Process-affected materials contain low levels of inorganic compounds including aluminium, nickel, vanadium, iron, barium, lithium, molybdenum, and boron (Chemistry data provided by Dr. Mike MacKinnon, Syncrude). Addition of sodium hydroxide during the extraction process also makes OSPM slightly alkaline (pH between 8 and 9).

1.2.4 Toxicity of Oil Sands Process Materials

Tables 1.1, 1.2 and 1.3 summarize the data generated by a number of studies focusing on the toxicity of oil sands waste products. In early research, laboratory bioassays were conducted to characterize the effects of OSPW on common aquatic test organisms such as the phosphorescent bacteria *Vibrio fischeri* (Microtox bioassay) and the water flea *Daphnia magna.* Fresh OSPW was found to be acutely toxic to both organisms (MacKinnon and Retallack, 1982, MacKinnon and Boerger, 1986). These laboratory experiments were followed by research conducted in the field, using the OSPM-affected wetlands and test ponds constructed by Syncrude and Suncor (see section 1.2.2) as mesocosms.

To date, nearly all research investigating the effects of OSPM on biota has focused on aquatic organisms. Results from the studies conducted on experimental wetlands suggest that some species of benthic invertebrates (Table 1.1) can thrive in OSPM-sites, forming communities with low species diversity but generally not causing changes in total biomass (Whelly, 1999, Bendell-Young et al., 2000, Ganshorn, 2002). Amphibians (Table 1.3) proved to be the most sensitive to OSPM and did not survive in experimental wetlands (Pollet and Bendell-Young, 2000). Studies with fish (Table 1.2) showed that the toxic response varied considerably between species, life stages and study sites (Peters, 1999, van den Heuvel et al., 1999 a & b, Bendell-Young et al., 2000). In birds (Table 1.3), the growth of nestling tree swallows and mallard ducklings was compromised on OSPM wetlands, but survival and reproductive success were not overtly affected (Smits et al., 2000, Gurney et al., 2005).

Table 1.1 Toxicity of Oil Sands Materials and Water (OSPW and OSPM) to invertebrates

Test organisms	Exposure	Effects	Reference
Water fleas (*Daphnia magna*)	Fresh OSPW (laboratory)	LC_{50} = 980 ml of OSPW/ L	Mackay and Verbeek, 1993
Chironomids	OSPM-wetlands (Syncrude & Suncor)	No effects on rates of oviposition, number of adults caught, or incidence of mouthpart deformities	Whelly, 1999
Chironomus tentans	Fresh OSPW (laboratory, 14 days)	Reduction of growth and survival in assays with concentrations of OSPW >50%	Whelly, 1999
Benthic invertebrates	OSPM-wetlands (Suncor)	Decreased community diversity, similar or increased density and biomass. No mouth parts deformities in chironomids. No mutagenic potential (Mutatox genotoxicity test)	Bendell-Young et al., 2000
Chironomids (Tanypodinae)	OSPM-wetlands (Syncrude & Suncor)	Greater density and secondary production (biomass) in OSPM-wetlands	Ganshorn, 2002
Zoobenthos	OSPM-wetlands (Syncrude & Suncor)	Decreased community diversity, similar biomass in young OSPM-wetlands	Leonhardt, 2003

Table 1.2. Toxicity of Oil Sands Materials and Water (OSPW and OSPM) to fish

Test organism	Exposure	Effects	Reference
Rainbow trout fingerlings	Fresh OSPW (laboratory)	LC_{50} = 125 ml of OSPW/ L	Mackay and Verbeek, 1993
Japanese Medaka embryos	Water from Syncrude OSPM-wetlands (laboratory)	Incidence of mortality and deformity increased with the concentration of OSPW	Peters, 1999
Yellow perch	OSPM-wetlands (Syncrude)	Decreased survival in ponds with high NA concentrations. Larger/heavier fish in OSPM-ponds.	van den Heuvel et al., 1999a
Yellow perch	OSPM-wetlands (Syncrude)	Increased EROD activity and increased PAH equivalents in bile. No effects on steroid hormones or gonadal growth.	van den Heuvel, 1999b
Fathead minnow and stickle back	OSPM-wetlands (Suncor)	Hematological changes (elevated hematocrit, depressed % leucocytes) and high mortality rates.	Bendell-Young et al., 2000
Young fathead minnows	Water from Syncrude OSPM-wetlands (laboratory)	No effects on fish dry weight. Decreased survival in one bioassay	Siwik et al., 2000
Young fathead minnow	OSPM-wetlands and laboratory (Syncrude)	Decreased weight gain during the field part of the experiment	Siwik et al., 2000
Yellow perch	OSPM-wetlands (Syncrude)	Gill lesions, fin erosions and skin tumors	van den Heuvel et al., 2000
Slimy sculpin and pearl dace	River adjacent to mining operations	Elevated EROD. No effects on steroid hormones, gonadal development, body mass and length	Tetreault et al., 2003
Fathead minnow eggs and larvae	OSPM from Suncor (laboratory)	Decreased hatching success, increased mortality, malformations and decreased size	Colavecchia et al., 2004

Table 1.3. Toxicity of Oil Sands Materials and Water (OSPW and OSPM) to amphibians and birds

Test organism	**Exposure**	**Effects**	**Reference**
Zebra finch	OSPW- (70 μL of CT water /day orally, from 6 to 9 days of age	No effects on T-lymphocyte immune response (PHA), hematocrit, WBC differential, body mass. Larger bursa of Fabricius.	Smits and Williams, 1999
Northern Canadian toad and wood frog tadpoles	OSPM-wetlands (Suncor)	Stunted growth in toad tadpoles. Very high mortality, decreased/absence of growth in wood frogs.	Pollet and Bendell-Young, 2000
Tree swallows	OSPM-impacted wetlands (Suncor and Syncrude)	Lower body weight (12 day-old) and increased EROD activity on the most impacted wetland. No effects on T-lymphocyte immune response (PHA).	Smits et al., 2000
Mallard ducklings	OSPM-impacted wetland (Suncor)	Lower body mass, smaller skeletal size	Gurney et al., 2005

1.3. NAPHTHENIC ACIDS

1.3.1 Naphthenic Acids in OSPW

Naphthenic acids are naturally present in bitumen and account for approximately 2% of raw petroleum by weight in the Athabasca basin (CEATAG 1998). Because of their high solubility in alkaline conditions (pKa ≈5), NAs are released from bitumen during the Clark Hot Water extraction process (see section 1.1.3). They become concentrated in the tailings ponds as the same water gets recycled multiple times during the extraction process (FTFC 1995). NAs are responsible for most of the acute toxicity of OSPW to aquatic organisms (Mackay and Verbeek 1993). Concentrations of NAs in tailings ponds can vary from 40-120 mg/L (Holowenko et al., 2002), but they typically range from 80-100 mg/L (Syncrude chemistry data, pers. comm. from M. MacKinnon).

1.3.2 Chemical Characteristics of NAs

Naphthenic acids are a complex group of saturated aliphatic, monocyclic and polycyclic alkanes with carboxylated aliphatic side chains of various lengths (Herman et al., 1994). They can be represented with the general chemical formula $C_nH_{2n+Z}O_2$, where n indicates the number of carbon atoms and 2n+Z the number of hydrogen atoms (Fig. 1.1). Z is an even and negative integer that specifies the homologous series to which the compound belongs. It represents the number of hydrogen atoms lost during the formation of a cyclic molecular structure. The absolute value of Z divided by 2 indicates how many rings are present in the molecule, e.g., compounds from the Z= -2 homologous series will have one ring, Z=-4 will have two rings and so on. Naphthenic acids with four or fewer rings dominate in tailings waters from Syncrude's main settling pond (Rogers et al., 2002b). Aliphatic naphthenic acids share some similarities with natural fatty acids, which also fit the formula $C_nH2_{n+Z}O_2$ (Z=0). However, acyclic NAs are highly branched, while fatty acids are not (Rudzinski et al., 2002, Clemente and Fedorak, 2005). The alkyl chain of NAs is hydrophobic, while their carboxyl group is polar and hydrophilic. This amphiphilic molecular structure gives NAs properties similar to those of surfactants (Schramm et al., 2000).

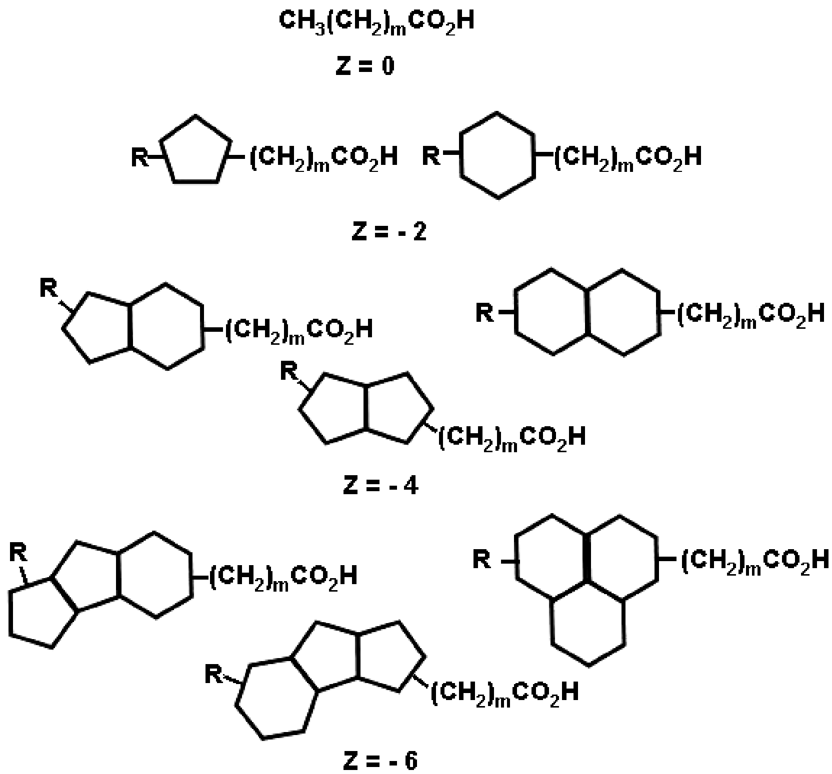

Fig. 1.1. Molecular structure of different naphthenic acids. "R" represents an alkyl group (C_nH_{2n+1}) and "m" refers to the length of the aliphatic side chain.
Source: Brient et al., 1995

1.3.3 Degradation and Fate

Under aerobic conditions, NAs undergo microbial biodegradation via the B-oxidation pathway, converting organic carbon into CO_2 (Herman et al., 1994). Naphthenic acids with lower molecular weight and fewer rings are more susceptible to biodegradation (Clemente, 2004). Little is known about the degradation processes in-situ, (i.e., within the tailings ponds), where anaerobic conditions predominate. In tailings water that has been transferred into shallow pits, total concentrations of NAs decrease over time. For instance, water from ten year old Demo Pond contains approximately 10 mg/L, while NA concentrations reach 80-110 mg/L in Mildred Lake settling basin, the main tailings pond from which Demo Pond's water originated.

1.3.4 Analytical Techniques

Because NAs are a complex mixture of compounds sharing similar properties, it is not possible to isolate specific molecules or to determine which ones are responsible for toxicity. Fourier transform infrared (FTIR) spectroscopy is the standard technique used by the oil sands industry to quantify naphthenic acids in water or oil. Measuring NAs in biological tissues has yet to be accomplished. FTIR measures the absorbance of the carbonyl groups of the carboxylic acids. Total NA concentrations can be measured with FTIR, but no information on the chemical composition of a mixture of NAs can be obtained because FTIR cannot distinguish different isomers. This is problematic because NAs are such a diverse family: for example, 37 types of NAs fit the formula $C_{10}H_{18}O_2$ (Z= -2), and they all have the same absorbance (Clemente and Fedorak, 2005). Recently, gas chromatography-electron impact mass spectrometry (GC-MS) methods have been used to describe the relative proportions of isomers in the mixture (Holowenko et al., 2002). Using a mathematical matrix based on the chemical formula of NAs, all the possible combinations of carbon numbers and Z family for specific molecular weights can be determined. The abundance (%) of the different ions can then be presented in 3-dimensional plots that provide a "fingerprint" of the NAs mixture (Fig. 1.2). Other analytical techniques used to measure NAs include gas chromatography (Jones et al., 2001), negative ion electroscopy ionization-mass spectrometry (Headley et al., 2002) and high performance liquid chromatography (Clemente et al., 2003).

1.3.5 Industrial Applications

Naphthenic acids recovered from petroleum are commercially available from companies such as Kodak, Merichem, Fluka and Pfaltz & Bauer. They serve as fungicides and flame-retardants in the textile industry, as emulsifiers in agricultural insecticides and petroleum-based chemicals, and as adhesion promoters in tire manufacture (Clemente and Fedorak, 2005). Naphthenic acids have also been added to oil paint to promote drying, and serve as lubricants and surfactants in the petroleum industry (Brient, 1995). Metal salts of naphthenic acids (copper and zinc) are increasingly used as wood preservatives to replace creosote (Brient, 1998). Naphthenic acids can also cause problems in petroleum refinery operations because they accelerate the corrosion of equipment (Brient, 1995).

1.3.6 Comparison of NAs from Oil Sands and Commercial Sources

Figures 1.2.1, 1.2.2 and 1.2.3 illustrate the chemical composition of different NA mixtures. Each bar in the three-dimensional plots represent one type (ion) of NAs, based on combinations of carbon number and Z families dictated by the chemical formula $C_nH2_{n+Z}O_2$, and the height of the bar shows the relative abundance (%) of that ion in the mixture. Naphthenic acids extracted from fresh OSPW (Fig. 1.2.1) contain few ions with more than 22 carbons, thus the bars for those carbon numbers are short. The fingerprint of NAs from aged tailings (Fig. 1.2.2) is quite different: ions with more than 22 carbons are proportionally more abundant, and a “valley” separates ions of ≤ 19 carbons and ≥ 22 carbons. The ions with 22-33 carbons have been referred to as the “22+ cluster” (Holowenko et al., 2002). Since their relative increase is accompanied by a relative decrease of ions with ≤ 19 carbons as tailings mature, it has been concluded that NAs of lower molecular weight (with lower carbon numbers) are degraded more readily than those with higher molecular weight (Clemente, 2004). Naphthenic acids from Kodak (Fig. 1.2.3) and Merichem (data not shown) are nearly devoid of ions falling into the “22+ cluster”, making them comparable to NAs extracted from fresh tailings, but not from aged ones (Clemente et al., 2003).

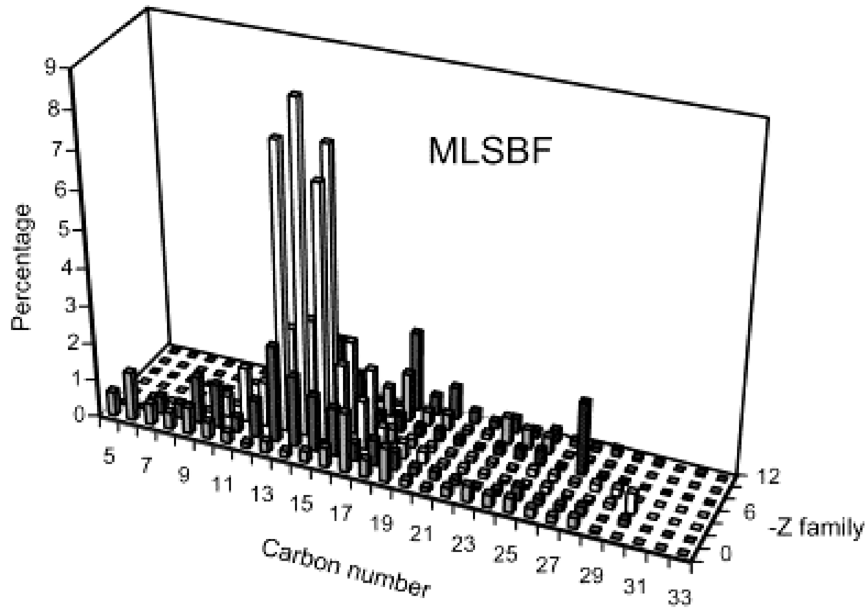

Fig. 1.2.1

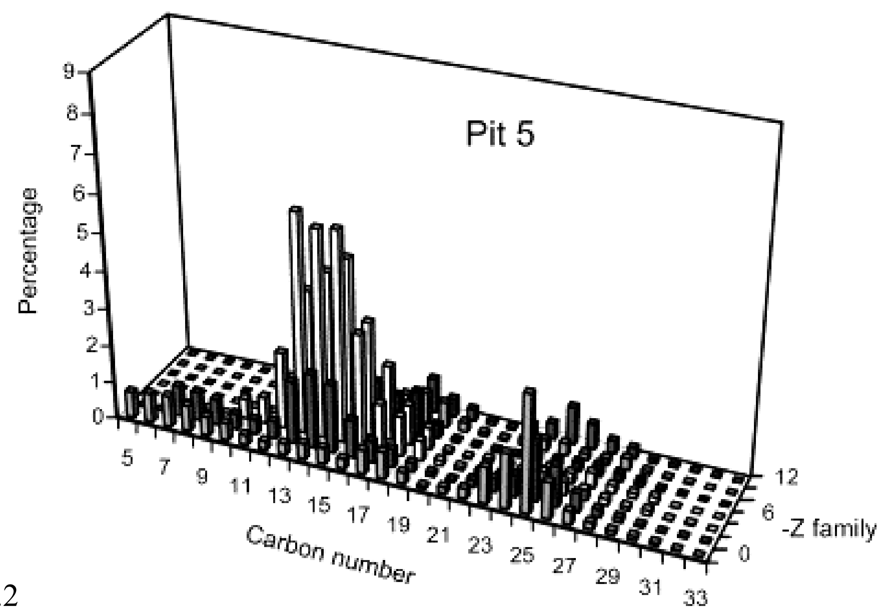

Fig. 1.2.2

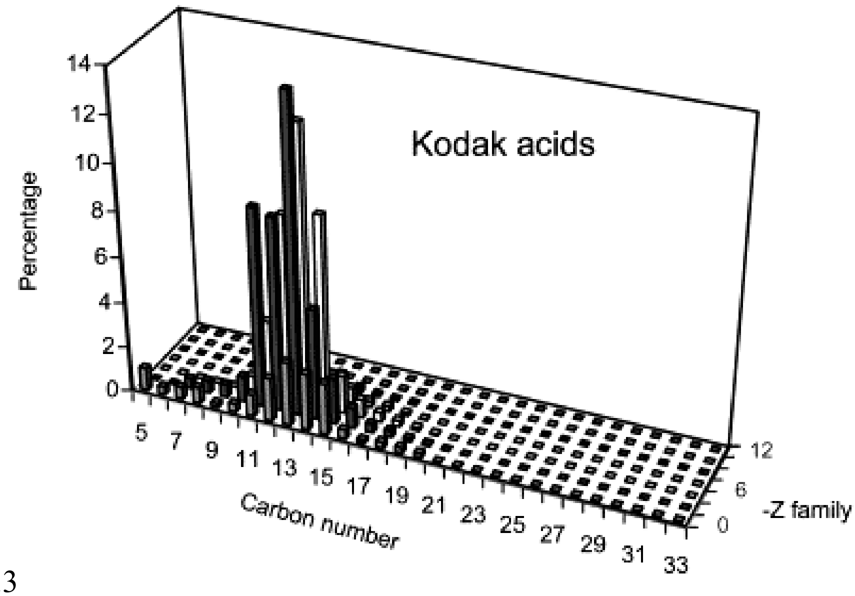

Fig. 1.2.3

Fig. 1.2. Distribution of ions in different naphthenic acids mixtures. Fig.1.2.1 shows NAs extracted from fresh tailings in Syncrude's Mildred Lake Settling Basin (MLSBF). Fig. 1.2.2 shows NAs from OSPW that have matured for 11 years in one of Syncrude's experimental test pond (pit #5). Fig 1.2.3 shows the fingerprint of NAs from Kodak™. *Figures from Clemente et al., 2003.*

1.3.7 Toxicity of Naphthenic Acids

The major findings from studies on NA toxicity are summarized in tables 1.4 and 1.5. Please note that the information we reported in Table 1.5 about the study from Maizelis et al., 1980, was cited by Rogers et al., 2002a in their article. Details about Maizelis' study were still unavailable at this time because their work was published in a Russian journal.

To date, research has been mostly limited to acute exposure studies to establish LD_{50} and LC_{50} for fish and laboratory rodents. Information on chronic toxicity is scarce. No studies have investigated the uptake, metabolism and elimination of NAs in biota. There are no published data on the toxicity of NAs to birds. Most of the above research on fish and rodents has been conducted using commercially available preparations of naphthenic acids rather than NAs extracted from OSPW. Since the composition of the NAs mixture highly depends on its geophysical source and changes over time, it is difficult to extrapolate the results from these studies to the actual situation on oil sands reclaimed sites. As mentioned in section 1.3.6, commercially available NAs show fingerprints relatively similar to that of NAs extracted from fresh tailings water, but are different from NAs that have matured in experimental test pits.

Nevertheless, these studies provide baseline data for comparing the toxicity of NAs with that of other chemicals. The LD_{50} studies with rodents show that acute toxicity of NAs to mammals is relatively low, and is comparable to that of table salt (Casarett and Doull, 1996). Only one study investigated chronic toxicity of NAs to mammals, and results suggest that this is much more of a problem than acute toxicity. Rogers (2003) found that a dosage just 10 times greater than the highest expected environmental exposure on reclaimed wetlands was sufficient to elicit dramatic effects on reproduction and hepatic function. Chronic toxicity or reproductive toxicity studies have not yet been conducted with birds.

Table 1.4. Toxicity of naphthenic acids to aquatic biota

Test organism	NAs source	Exposure & Effects	Reference
Snail	Commercial	LC_{50} (96hrs) = 6.6-7.5 mg/L	Patrick et al., 1968
Bluegill sunfish	Commercial	LC_{50} (96hrs) = 5.6 mg/L	Patrick et al., 1968
Five Caspian fish species	Commercial	LC_{50} (96hrs) ranged from 25 mg/L (chum salmon) to 75mg/L (round goby and roach).	Dokholyan and Magomedov, 1983
Juvenile chum salmon	Commercial	LC_{50} (60 days) = 1.4 mg/L in juvenile chum salmon. LC_{0} (100% survival, 60 days) = 0.32 mg/L	Dokholyan and Magomedov, 1983
Sturgeon, roach, chum salmon	Commercial	Sturgeon and roach (0.5-5mg/L, 45 days): Increased then decreased leucocyte counts. Chum salmon (6-days): Decrease in muscle glycogen content at 5 mg/L; increase in muscle glycogen content at 0.5-1.0 mg/L	Dokholyan and Magomedov, 1983
Stickleback	Commercial	LC_{50} (96 hrs) = 5 mg/L	Davis, 1992
Phytoplankton	OSPM-wetlands (Syncrude)	Shifts into community composition associated with increasing NAs concentrations. No effects on total biomass	Leung et al. 2001, 2003

Table 1.5. Toxicity of naphthenic acids to laboratory rodents

Test organisms	**NAs source**	**Exposure & Effects**	**Reference**
Rat	Commercial	I.P. injection (1 ml, 1% aqueous solution, every second day, 21 days): elevated liver glycogen	Khanna et al., 1972
Mouse	Commercial	LD_{50} (oral exposure) = 3.55 g/kg. Central nervous system depression, diarrhoea, convulsions, respiratory arrest	Pennisi and Lynch, 1977
Rat	Commercial	I.M. injection (0.15 g/kg, daily, 10 days): increased vascular permeability of capillaries	Maizelis et al., 1980
Rat	Commercial	LD_{50} (oral exposure) = 3g/kg of body weight	Lewis, 2000
Rat	Extracted from MLSB	Acute exposure (300 mg/ kg orally): anorexia, pericholangitis, cerebral haemorrhage, cardiac periarteriolar necrosis. Chronic exposure (60 mg/kg/d, 90 days): decreased growth, depressed plasma cholesterol, excessive hepatic accumulation of glycogen.	Rogers, 2003
Female rat	Extracted from MLSB	Chronic reproductive toxicity study (60 mg/kg/d, 7 weeks): highly compromised reproductive success. NA exposure associated with impaired embryonic implantation.	Rogers, 2003

1.4. STUDY SITES

The study was conducted on one reference site and three experimental reclaimed wetlands (containing OSPM) located on Suncor's and Syncrude's leases (57º 00' N, 111º 30 E), from late May to mid July of 2003 and 2004. Wetlands exhibiting various degrees of maturation and bioremediation of the OSPM were chosen to illustrate the different stages of reclamation. Naphthenic acid (NAs) concentrations in water (collected in 2003) were obtained from Golder Associates (2004) and from Dr. Mike MacKinnon (Syncrude's Edmonton Research facility). Concentrations of PAHs in three of those reclaimed wetlands have been described previously (Smits et al., 2000) and data pertinent to our study sites are reproduced here.

1.4.1 "Consolidated Tailings" (CT, Suncor)

Consolidated Tailings (CT) wetlands, on Suncor's lease, are adjacent to a 300 ha tailings settling basin (Fig.1.3). It was created in 1999 by flooding a 52 ha area with consolidated tailings, which contained about 50% water and 50% solids (sand, fine tailings and bitumen) (Golder Associates, 2004). Parts of the site were devoted to terrestrial reclamation and covered with muskeg, while the remaining low lying areas became wetlands. The wetlands receive tailings water from the settling basin through a discharge pipe. Because of the fresh input of OSPW, this site is representative of early stages of reclamation. Concentrations of NAs are higher in CT wetlands than at the other sites (Table 1.6). Concentrations of PAHs in sediments have not yet been measured on this site. Nevertheless, since this is the newest reclaimed wetland and that concentrations of PAHs decease over time, they would likely be higher than on the other study sites. The wetlands areas also receive dyke seepage and local runoff water. Young white spruce (*Picea glauca*) now ranging from 1.5m to 2m-high have been planted on this site to test the success of revegetation strategies.

1.4.2 "Natural Wetlands" (NW, Suncor)

The Natural Wetlands on Suncor are adjacent to the same settling basin as CT wetlands (Fig.1.3). This 1.3 ha depression formed in 1984 following terrestrial reclamation of the site. Precipitation and water seeping through the dykes enclosing the tailings pond gradually filled the depression. Tailings water was pumped from the settling basin into NW between 1999 and 2001. This site is representative of maturing reclaimed wetlands, where microbial activities have led to some degree of bioremediation. Abundant shrub communities dominated by willow (*Salix* spp.) surround the wetland and mixed forest borders the study site (white spruce *Picea glauca*, balsam poplar *Populus balsamifera* and trembling aspen *Populus tremuloidies*).

1.4.3 "Demonstration Pond" (DP, Syncrude)

Demo Pond is a round 4-5 ha test pond that was constructed in 1993 (Fig. 1.3). It was filled with a 9 m deep layer of fine tailings, on top of which 2.5m of diverted local surface stream flow was added (Golder Associates, 2002). This is the oldest experimental site studied in this project and concentrations of NAs as well as PAHs have substantially decreased over time (Table 1.6). The Demo Pond site is nearly devoid of trees or shrubs, and grassy pastures surround the area.

1.4.4 "Poplar Creek" (PC, Reference Site)

Poplar Creek is a 2.5 km x 0.5 km reservoir located approximately 10 km south of mining operations (Fig. 1.3). It was created in 1975 when a river flowing through the area to be developed for mining had to be diverted prior to beginning operations. It does not contain any mine tailings. Concentrations of NAs and PAHs at this site are typical of those of natural lakes in the Athabasca region (Smits et al., 2000). A dyke and a concrete spillway form the southern shore of the reservoir. Apart from the white spruces that were planted in the vicinity of the spillway in the 70s' to replace those that were cut during the construction of the dyke, PC reservoir is surrounded by mature forest typical of the Central Mixedwood Subregion of the boreal forest (Beckingham and Archibald, 1996). It is dominated by white spruce, trembling aspen and balsam poplar.

Table 1.6. Concentrations of naphthenic acids (mg/L) in water and PAHs (ng/g) in sediments on the four study sites

	Consolidated Tailings Wetlands (CT)[a]	**Natural Wetlands (NW)**	**Demo Pond (DP)**	**Poplar Creek Reservoir**[b] **(PC)**
NAs[c]	68.0	51.9	10.3	0.3
Parent PAHs[d]	_	207.7	140.1	81.5
Alkylated PAHs[e]	_	2273.1	1010.0	175.9

[a] Concentrations of PAHs in CT wetlands were unavailable
[b] Reference site
[c] Source: measured in 2003. Concentrations from CT wetlands are from Golder Associates 2004 concentrations in NW, DP PC are from Dr M. MacKinnon's database, Syncrude Research Facility, 2004
[d] Source: measured in 1998, reported in Smits et al., 2000
[e] Total of C1-C4 naphthalenes, C1-C4 fluorenes, C1-C4 phenanthrenes/anthracenes, C1-C4 fluoranthenes/pyrenes and C1-C3 dibenzothiophenes; measured in 1998, summarized from Smits et al., 2000.

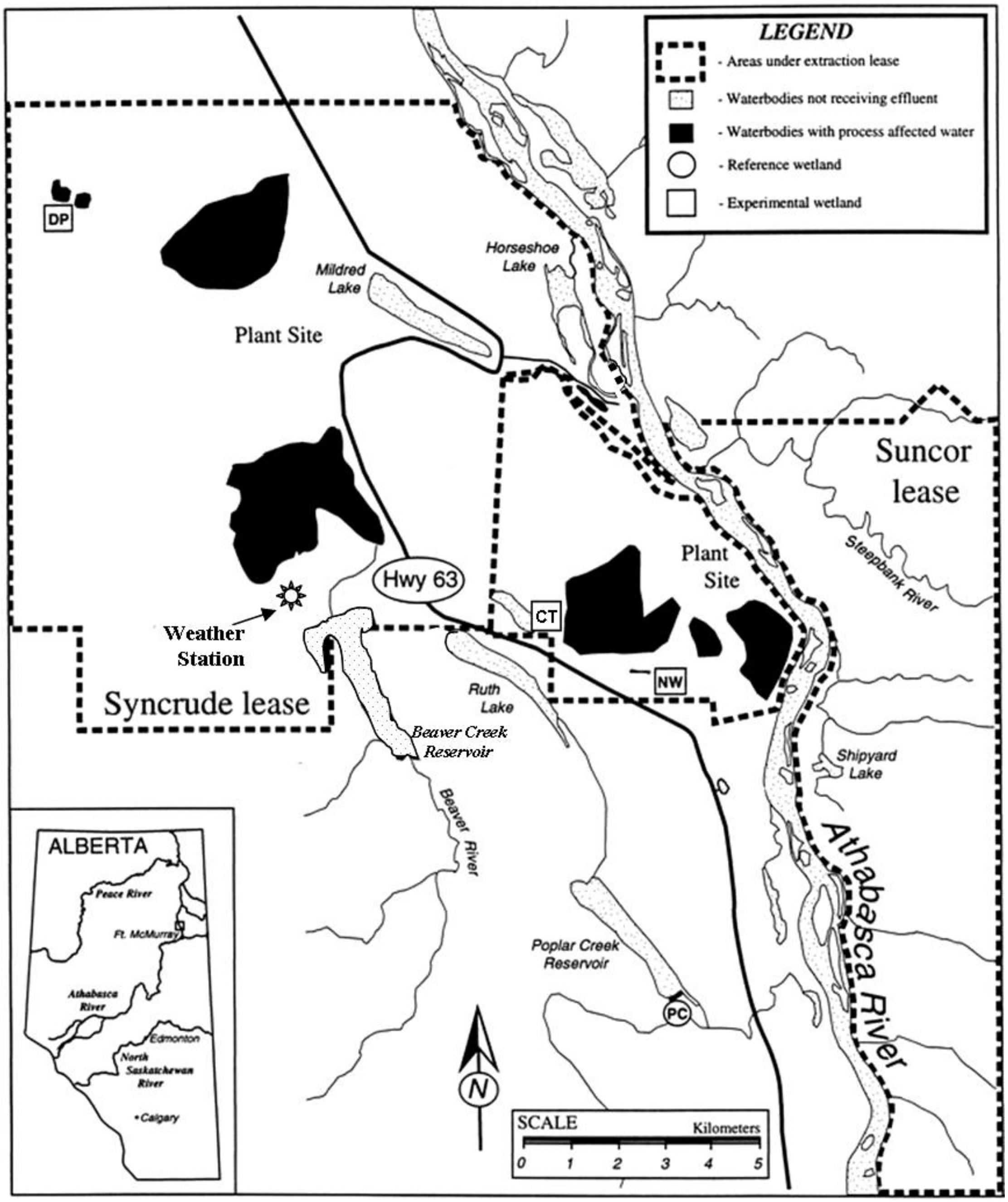

Fig. 1.3. Map of the study area, showing the mining leases, tailings ponds, study sites and meteorological station. PC=Poplar Creek (reference site), DP=Demo Pond (Syncrude), NW=Natural Wetlands (Suncor), CT=Consolidated Tailings Wetlands (Suncor).

Adapted from Smits et al., 2000.

1.5. STUDY ORGANISM

1.5.1 Tree Swallows Sentinels of Environmental Health

In the last 25 years, the tree swallow (*Tachycineta bicolor*) has been the focus of such a diversity of research, ranging from mating systems to nest-building behavior and climate change, that it has been deemed a “new model organism” (Jones, 2003). Because tree swallows readily use nest boxes which can be placed on target study areas, and because they feed mainly on insects with aquatic larval stages which develop in contaminated water and sediments, they are an effective indicator species of environmental health (Bishop et al., 1999, McCarty and Secord, 1999, Smits et al., 2000). An extensive review of the ecotoxicology literature focusing on tree swallows can be found on the website of the USGS Patuxent Wildlife Research Center (USGS 2005).

1.5.2 Biological Characteristics of the Tree Swallow

Adult *Tachycineta bicolor* (Passeriforme:Hirundinidae) weigh 18-23 g and reach about 14 cm from the tip of the bill to the end of the longest tail feather (Robertson et al., 1992). The upper body of males and adult (≥ 2 year old) females is iridescent blue, while one year-old females and juveniles bear duller brownish plumage, and underparts are always white (Hussell 1983). Tree swallows are abundant and widely distributed throughout North America (Fig.1.4). The northern limit of their breeding range approximately coincides with the tree line (Erskine, 1977). They spend the winter on the southern coast of the Gulf of Mexico or in Central America (Butler, 1988).

Fig. 1.4. Breeding range of the tree swallow in North America.
Cornell Lab of Ornithology(*http://www.birds.cornell.edu*)

1.5.3 Life History

Tree swallows prefer open landscape rather than forested areas. They are cavity nesters and will typically be found around lake shores, wetlands and field margins where snags have been excavated by woodpeckers (Robertson et al., 1992). Adults usually forage within 100m from their nest (Wayland et al., 1998, McCarty, 2002). Tree swallows are mainly monogamous and raise one brood over the summer. Clutch size can vary from two to eight eggs but is most commonly 4 to 7 eggs (Stutchbury and Robertson 1988). Previous research on the oil sands area showed mean clutch size ranging from 6.3 (1997) to 6.9 (1998) eggs on Poplar Creek (Smits et al., 2000). Young females (first breeding season) or females initiating clutches later in the season often have smaller clutch size and poorer reproductive success (Stutchbury and Robertson, 1988). Incubation lasts for 14-15 days, starting on the day the penultimate (second last) egg is laid, and most clutches hatch over a 1-3 day period. Nestlings leave the nest between the ages of 18 and 22 days (Burtt 1977). They do not usually return to the cavity once they have departed, but do receive feedings from their parents for several days after leaving the nest (Robertson et al., 1992). The average life span of tree swallows is 2.7 years, but individuals may survive up to 8-11 years (Hussell, 1982, Butler, 1988).

1.5.4 Diet

Only one study analyzed the stomach contents of adult tree swallows (Beal, 1918). The diet contained 41% Diptera, 14% beetles, 6 % ants and about 20% vegetable matter such as bayberries. The remaining research was conducted on nestlings. Food boluses are commonly recovered from the crop of nestlings equipped with a collar to prevent swallowing (McCarty, 2002). Another technique previously used to collect dietary samples involved interesting-looking tree swallow puppets constructed with the skin of dead nestlings (McCarty and Winkler, 1991), but this method never gained popularity. Smits et al. (2000) collected boluses from nestlings on three of our study sites and found them to contain 84% insects of aquatic origin. Among those, Tabanidae (horseflies, 33%), Ephemeroptera (mayflies, 22%), Chironomidae (midges, 13%) and Syrphidae (hoverflies, 11%) were the most common in dietary samples. One study near Ithaca (NY) found tree swallows to prefer insects > 3mm long over smaller ones, occasionally capturing preys measuring up to 60 mm (McCarty and Winkler, 1999b).

1.6 OTHER AVIAN SPECIES ON THE OIL SANDS

Avian communities that could be affected by the habitat destruction associated with open pit mining represent a wide range of boreal species. This section primarily focuses on species that have been found on reclaimed wetlands. Shortt (1988) conducted a census of birds on waterbodies in and around Syncrude mining sites. He surveyed the main tailings pond (Mildred Lake Settling Basin), two uncontaminated lakes (Mildred Lake and Beaver Creek reservoir) as well as a number of small ponds scattered around the extraction plant. These water bodies supported an average of 164 birds per survey and 44 different species were observed. Diving ducks made up the largest number of species and comprised 34% of all birds seen. Buffleheads and ring-necked ducks dominated this group. A census of woodland species breeding on Syncrude leases (Murray et al., 1988) reported a total of 59 species with a density of 25 birds per kilometer of transect. Most abundant species included Swainson's thrush and ruby-crowned kinglet in black spruce habitat, western tanager and bay-breasted warbler in spruce-aspen habitat, as well as ovenbird, red-eyed vireo and Canada warbler in aspen-alder habitat. In an extensive wildlife survey conducted on Suncor leases (Golder Associates, 2003), the Tennessee warbler was the most commonly recorded songbird. A number of species of special concern (i.e., At Risk, May Be at Risk, Sensitive) were detected including the bay-breasted warbler, Cape-May warbler, pileated woodpecker and western tanager. All bird species observed or heard at the Hummock-wetlands area which is adjacent to our study site Natural Wetlands are listed in annex 1 (Golder Associates, 1997).

CHAPTER 2

Effects of Oil Sands Tailings Compounds and Harsh Weather on Reproduction, Mortality Rates, Growth and Detoxification Efforts of Tree Swallows

2.1 ABSTRACT

Oil sands mining companies in Alberta, Canada, are evaluating the feasibility of using wetlands to detoxify Oil Sands Process Materials (OSPM) as a reclamation strategy. Reproductive success, nestling growth, survival and hepatic biotransformation activity (ethoxyresorufin-O-deethylase) were measured in tree swallows on OSPM-wetlands and meteorological data were obtained from Syncrude. In 2003, an abrupt temperature plunge synchronized with persistent rain triggered a widespread nestling die-off. Reproductive success on the two most process-affected wetlands was among the lowest ever reported for tree swallows. This was linked to very poor nestling survival on reclaimed sites. Mortality rates of nestlings reached 89% and 100% on the two newest OSPM sites, while on the reference site they did not surpass 50%. In 2004, weather was less challenging. Mortality rates were low and did not vary among sites, but 12 day-old nestlings on OSPM-wetlands had higher ethoxyresorufin-O-deethylase (EROD) activity and weighed less than those on the control site. These results indicate that compared with reference birds, nestlings from OSPM-impacted wetlands may be less able to withstand additional stressors, which could decrease their chances of survival after fledging.

2.2 INTRODUCTION

The Oil Sands of northeastern Alberta, Canada, cover over 140,000 km^2 and represent one of the world's largest reserves of crude oil (Alberta Department of Energy 2005). Separation of the heavy crude oil from sand is accomplished by the addition of large volumes of hot water. Once the bitumen has been extracted, residual sand, clay, and process water, along with organic and inorganic contaminants are diverted to vast settling ponds on the mining sites (Mikula et al., 1996). These tailings are accumulating at the rate of ~10^5 m^3/day (Madill et al., 2001), and since there is currently no discharge of the mining effluents from the mine leases, as much as 1 billion m^3 of Oil Sands tailings will require detoxification and reclamation upon mine closure (FTFC 1995).

As a result of leaching from the ore and the addition of chemicals during proceessing, the water used in the extraction of bitumen (Oil Sands Process-Affected Water, OSPW) becomes contaminated with polycyclic aromatic hydrocarbons (PAHs) and dissolved organic chemicals such as naphthenic acids (Mikula et al., 1996). Process-affected water has been shown to be toxic to plankton communities (Leung et al., 2003), fish (van den Heuvel et al., 2000) and amphibians (Pollet and Young, 2000). Reclamation of the mining areas has included the creation of artificial water bodies (wet landscape reclamation) to receive aging tailings, which are capped with a layer of fresh water (Gulley and MacKinnon, 1993). Most of the acute toxicity to aquatic organisms should be mitigated by natural processes such as microbial biodegradation, but long-term ecological impacts may still occur (Lai et al., 1996).

To investigate the feasibility, efficiency and sustainability of the wet landscape reclamation strategy, the two largest Oil Sands mining companies, Syncrude Canada Ltd. and Suncor Energy Inc., have constructed in the past 15-20 years a series of experimental wetlands and test ponds, which were partly filled with mine tailings to mimic future reclamation plans. Tree swallows (*Tachycineta bicolor*) nesting around these wetlands were designated as upper trophic level bioindicators of environmental contamination, because insects of aquatic origin accounted for 84% of their diet in a previous study (Smits et al., 2000). Tree swallows are an effective model species, and they have been widely used in environmental impact assessments (reviewed by Jones, 2003). In most of these assessments, reproduction has been an important endpoint. A number of studies reported a reduction in reproductive performance on sites affected by chemicals of anthropogenic origin (Bishop et al., 2000a, Custer et al., 2003, McCarty and Secord, 1999), but others did not detect measurable effects (Elliott et al., 1994,, Custer et al., 1998, Wayland et al., 1998, Bishop et al., 1999, Harris and Elliott, 2000, Gerrard and St-Louis, 2001). The biological effects on the swallows were often surprisingly low considering the degree of exposure to contaminants. However, most of these field studies were conducted under favorable meteorological conditions. Birds may respond differently to chemical exposure if they are simultaneously subjected to stressful environmental conditions. The impact of inclement weather on the reproduction of birds nesting on contaminated sites was investigated in only one of these studies (Custer et al., 2003).

In the current study, meteorological conditions were measured on the study sites and population dynamics of tree swallows were monitored during two summers with different weather patterns. During both years, we investigated the relationship between exposure to OSPM and reproductive success of adult tree swallows, as well as growth and survival of nestlings. Since organic pollutants from other industry sources are known to induce hepatic detoxification enzymes, we also measured ethoxyresorufin-*o*-deethylase (EROD) activity in nestlings as an indicator of exposure to chemicals that are present in the OSPM.

2.3 METHODS

2.3.1. Study Sites

The study was conducted on one reference site and three wetlands containing OSPM located on Suncor's and Syncrude's leases (57º 00' N, 111º 30 E), from late

May to mid July of 2003 and 2004. Please consult Chapter 1 for a detailed description of the study sites.

2.3.2. Meteorological Data

Meteorological data were recorded at Syncrude's meteorological station, which has a central location relative to the study sites (Chapt. 1, Fig. 1.3). Data presented here are average temperatures, mean daily precipitation and cumulative rainfall. To examine the effects of weather on nestling survival, I used data from June 18th, which was the earliest an egg hatched in both years of study, to July 11th, when the last nestling would have fledged (i.e. the latest a nestling reached the age of 14 days in both years of study).

2.3.3 Tree Swallows

A total of 92 nest boxes were monitored on all study sites, with 26 boxes on Poplar Creek reference site (PC), 20 on Demonstration Pond (DP), 25 on Consolidated Tailings (CT) and 21 on Natural Wetlands (NW). Tree swallow activity was monitored from May 25th to July 15th. Nest boxes were at first inspected daily to determine clutch initiation date and clutch weight. Clutch weight was measured on that day, i.e., the third day with the same number of eggs in the nest. Since females occasionally interrupted laying for one day or two, a clutch was considered complete when no new egg was laid after three days. Females were aged according to plumage characteristics (Hussell, 1983). Nests were left undisturbed during the 14 day incubation period and then monitored daily to determine hatch date and hatching success (number of eggs hatched/number of eggs laid). Nestlings within a brood were uniquely identified with different color combinations on the claws. Nestling weight and wing length (carpus to end of longest primary feather) were measured on days 6 and 12 (hatch day = day 1). Egg and nestling mass were measured to the nearest 0.05g using a Pesola spring balance, wing length was measured to the nearest 0.1cm. In 2003, the number of nestlings that survived to day 12 was used to calculate fledging success (number of fledglings/eggs hatched) and nest success (number of fledglings/eggs laid). In 2004, this was changed to day 14 because of delays in obtaining NAs for the experimental exposure study. Nestlings that disappeared before 6 days of age were considered “dead” (they were small enough that they could have been removed by a parent after death). Nestlings that disappeared after 6 days of age were considered lost to predators and were excluded from the analysis of reproductive performance.

2.3.4 EROD Activity

In 2004, one to three 14-day-old nestlings per nest were collected to measure hepatic EROD activity. Nestlings to be collected were randomly chosen within broods (nestling ID numbers were placed in a hat and picked at random). Nestlings were anesthetized with inhalation of halothane (Halocarbon Laboratories, River Edge, NJ, USA) and euthanized by cervical dislocation. Liver was removed and the left lobe (0.2-0.3 g) was frozen in liquid nitrogen (-170 °C) within 5 minutes of collection. After the end of the field season, samples were transferred to a –70 °C freezer until further analysis. In October, liver samples were thawed and homogenized with 1 ml of KCl (0.15M) – HEPES (0.02M) buffer to isolate microsomes. Microsomes were separated from other cellular components after selective ultracentrigugation. Homogenates were centrifuged at 12.500 rpm (20 minutes) then supernatant was transferred into clean tubes and centrifuged again at 38,500 rpm (60 minutes). The pellet resulting from that centrifugation contained the microsomes. Supernatant was discarded, the pellet was resuspended in homogenization buffer and centrifuged again at 38,500 rpm to separate remaining cellular debris. Finally, the microsomal pellet was homogenized in resuspension buffer (0.05 M tris, 1nM EDTA, 20% glycerol; pH7.4) and stored at –70 °C in cryovials until measurement of enzymatic activity.

Enzymatic activity was quantified using an adaptation of the Canadian Wildlife Service’s protocol (Trudeau and Maisonneuve, 2001). Microsomes samples were placed in 96-well plates kept on ice (NUNC-immuno plates, Nalge Nunc International, Rochester, NY, USA). Total volume in the wells was 140 uL. Sample wells contained 75-200 ug/ml of microsomal proteins, 1 uM ethoxyresorufin (Molecular Probes R-352, Invitrogen Canada, Inc., Burlington, ON, Canada) and 0.5 mM NADPH (Sigma-Aldrich Canada Ltd. N-6505, Oakville, ON, Canada). The reaction was allowed to proceed for 10 minutes at 39°C, then was stopped by adding 60 uL of solution containing 600 ug/ml of fluorescamine (acetonitrile: AXO145, BDH Inc. Toronto, ON, Canada, fluorescamine: Sigma-Aldrich F-9015). Fluorescence of resorufin product was measured using 390 nm excitation / 460 nm emission filters while fluorescamine, indicating the amount of protein, was measured using 530 nm excitation / 590 nm emission filters (96-wells fluorometer, MFX Microtiter Plate reader, Dynex Technologies Inc., Chantilly, VA, USA). Enzymatic activity was expressed as pmol/min/mg microsomal protein.

2.3.5 Statistical Analysis

Data from nestlings of one brood are very likely to be correlated, as nestlings from one brood cohabit in a common environment, receive food from the same parents, inherit genetic traits from those parents, etc. Because most standard statistical tests are based on the assumption that all observations must be independent, using those tests with individual nestlings as experimental units could lead to spurious significant results. One approach used to deal with the lack of independence between such samples has been to convert the data from each nest into a single observation, e.g., to average measurements of all nestlings within a brood and use "nest averages" for the statistical analysis. However, this substantially diminishes statistical power. Multilevel models are designed to analyze hierarchical data (clustered data). For instance, individuals clustered within households, clustered within different geographical areas can be surveyed, using statistical equations that properly include all the appropriate dependencies (Hox, 2002). This approach has recently been used in ecological research with nestling birds (Simon et al., 2004). In this study, we used linear mixed models (one technique of multilevel analysis) to detect associations between SITE (DP, PC, NW, CT) and continuous outcomes (e.g. weight, wing length). Detailed explanations about mixed models would be beyond the scope of this thesis, so the reader is invited to consult Dohoo et al., 2003. Briefly, individual nestlings were considered as units clustered within broods and the computer was programmed to account for the lack of independence among chicks from the same nest by adding a random effect for "nest" in each model (PROC MIXED, SAS for Windows version 8.02, SAS Institute Inc., Cary, NC.). The associations between SITE and discrete outcomes were similarly (controlling for clustering by nest) analyzed using generalized estimating equations (PROC GENMOD, SAS for Windows version 8.02). A Poisson distribution with log link function was used for modeling count outcomes (e.g., clutch size, brood size, fledglings/nest). A binomial distribution with logit link function was used for dichotomous data such as reproductive success (e.g., for hatching success, egg hatched=1 or egg unhatched=0). The effects of site on nestling mortality were analyzed using logistic regression models, where each nestling was assigned a binary response variable (dead=0, survived=1). Detailed explanations on the use of logistic regression in the statistical analysis of "success versus failing" data (such as reproductive success) can be found in Petrie and Watson, 1999.

Because few breeding pairs nested on CT in 2003, sample size was too small to carry out statistical tests on fledging success, nest success, number of fledglings/nest and nestling mortality on this site, thus data from CT were excluded from statistical comparisons. In order to not completely lose the information from this site and allow some assessment of the situation on CT, the combined data from CT and NW is also presented in this chapter. Combining the data was based on the facts that CT and NW were both located on Suncor's lease, were geographically very close to each other (less than one km), were the two newest reclaimed sites, and because concentrations of NAs and presumably PAHs were high in both sites. Mostly, reproductive success was extremely low on both sites and mortality rates were similarly very high on both sites. The lumped data is identified as "Suncor". It should be noted that this was based on arithmetic means and it was not statistically proven that there were no differences (in endpoints) between NW and CT (because CT data could not be analyzed alone). Thus, comparisons using the lumped data are always presented along with those from which CT data were excluded, so the *P* values of CT excluded versus CT-NW lumped can be compared and the effects of CT as a site can be roughly estimated.

Other confounding factors for reproductive success and nestling growth include female age, brood size and hatch date. Clutches laid later in the season or reared by younger females tend to be less successful, and chicks from larger broods may have slightly reduced growth rates (Robertson and Rendell 2001). Female age, hatch date and brood size were therefore included in the models as covariates when $P \leq 0.05$ (or inclusion of the factor in the model changed the P value by more than 10%).

2.4. RESULTS

2.4.1 Tree Swallow Reproduction

A total of 53 and 50 breeding pairs nested on the study sites in 2003 and 2004, respectively. Female age and brood size were not associated ($P > 0.05$) with any measures of reproductive success (hatching, fledging and nest success) or reproductive performance (clutch size, brood size, number of fledglings) in either year. In 2003, the date of clutch initiation (in julian days) was negatively associated with most parameters of reproductive success. Clutch size ($P = 0.008$), brood size ($P = 0.005$) and numbers of fledglings (P= 0.04), as well hatching success ($P = 0.02$) and nest success ($P = 0.03$) decreased as the season progressed. However, fledging success was not associated with clutch initiation date ($P = 0.3$). In 2004, clutch initiation date did not significantly affect any reproductive parameter ($P > 0.05$).

Clutch size ($P = 0.6$) and brood size ($P = 0.3$) did not differ between sites in 2003 (Table 2.1). However, on reclaimed wetlands, nests contained very few nestlings by the time of fledging. This was particularly striking on the two newest OSPM sites: on CT, no nestling survived to d12 and on NW, less than one nestling /per nest reached the 12 days of age. Differences between the number of fledglings in nests from NW and PC were highly significant ($P = 0.006$). CT wetlands were excluded from individual site comparisons due to an insufficient number of active nests. Differences between PC and combined data from Suncor (CT and NW) were similarly significant ($P = 0.006$). There was no difference in the number of young fledged between DP and PC (*P* =0.09). Hatching success (chicks hatched/eggs laid) was similar across sites (*P* = 0.4) (Table 2.2). Fledging success (0-12%) and nest success (0-7%) were very poor on the newest reclaimed sites (Table 2.2). Compared with PC, fledging success (chicks fledged/ chicks hatched) and nest success (chicks fledged/eggs laid) were lower on NW (*P* = 0.02 and *P* =0.007, respectively). Data from CT had to be excluded from site comparisons because of the small sample size, but fledging success (*P* = 0.003) and nest success (*P* = 0.01) on Suncor sites (combined data) were lower than on PC. In DP nests, fledging success was similar to control nests (*P* =0.6). Nest success was, however, significantly lower on DP than on PC sites (*P*=0.04).

The 2004 breeding season was more productive for tree swallows and differences between reclaimed wetlands and the control site were not as dramatic as in 2003. Clutch size was similar across sites (*P* = 0.4), but brood size was smaller on DP than on PC (*P* =0.01), and fewer chicks reached fledging age on DP than on PC (*P*=0.02) (Table 2.1). There were no differences among sites in hatching success (*P* = 0.1), fledging success (*P* =0.5) or in nest success (*P* = 0.07) (Table 2.2).

Table 2.1. Reproductive performance (mean ± SD) of adult tree swallows on the four study sites in 2003 and 2003

		Poplar Creek (PC)[a]	**Demo Pond (DP)**	**Natural Wetlands (NW)**	**Consolidated Tailings Wetlands (CT)**
Clutch size	2003	6.3 ± 0.8 A n=26	6.0 ± 1.0 A n=12	5.3 ± 0.7 A n=10	4.8 ± 1.9 A n=5
	2004	6.7 ± 0.7 A n=22	6.4 ± 0.8 A n=14	6.2 ± 0.8 A n=5	6.3 ± 0.7 A n=9
Brood size[b]	2003	5.9 ± 0.9 A n= 26	4.3 ± 2.5 A n= 12	3.6 ± 2.0 A n=10	3.2 ± 2.9 A n=5
	2004	6.3 ± 1.1 A n=22	4.3 ± 2.5 B n=14	5.6 ± 1.1 A n=5	6.1 ± 0.8 A n=9
Fledglings /nest	2003	3.2 ± 2.6 A n= 26	1.8 ± 2.7 B n=12	0.4 ± 1.1 B n=7	0 ± 0 * n=3
	2004	6.3 ± 1.1 A n=16	4.3 ± 2.5 B n=12	5.6 ± 1.1 A n=5	6.0 ± 0.8 A n=8

* = Sample size too small for comparison, n= number of nests
[a] Reference site
[b] Initial brood size (at hatch)
Different letters indicate a statistical difference among sites ($P \leq 0.05$).

Table 2.2. Reproductive success (mean ± SD) of adult tree swallows on the four study sites in 2003 and 2004.

		Poplar Creek (PC)[a]	**Demo Pond (DP)**	**Natural Wetlands (NW)**	**Consolidated Tailings Wetlands (CT)**
Hatching success[b]	2003	92.8 ± 8.8 A n= 26	70.5 ± 39.9 A n= 12	67.0 ± 37.0 A n=10	53.8 ± 49.5 A n=5
	2004	94.2 ± 10.8 A n= 22	69.5 ± 39.6 A n= 14	89.8 ± 9.5 A n= 5	96.8 ± 9.5 A n=8
Fledging success[c]	2003	55.2 ± 42.5 A n=26	44.0 ± 49.7 A n=10	12.0 ± 26.8 B n=5	0 * n=1
	2004	96.8 ± 6.9 A n= 16	100.0 ± 0 A n= 9	93.3 ± 14.9 A n=5	100.0 ± 0 A n=8
Nest success[d]	2003	51.1 ± 39.6 A n= 26	25.9 ± 11.0 B n=12	7.1 ± 18.9 C n=7	0 ± 0 * n=3
	2004	92.3 ± 12.9 A n= 16	64.4 ± 40.7 A n=12	83.1 ± 11.7 A n=5	96.4 ± 10.1 A n=8

* Sample size too small for comparison, n= number of nests
[a] Reference site
[b] Hatching success (%) = eggs hatched/eggs laid per nest
[c] Fledging success (%) = chicks fledged/eggs hatched in nests where ≥ 1 egg hatched
[d] Nest success (%) = chicks fledged/eggs laid per nest
Different letters indicate a statistical difference among sites ($P \leq 0.05$).

2.4.2 Nestling Growth

In 2003, nestlings on day 6 (d6) post-hatch (hatch day = d1) weighed less (P = 0.02) and had shorter wing length (P =0.007) as the season progressed (nestling size was negatively correlated with hatch date), but there was no correlation between nestling size and hatch date at the age of 12 days (weight d12: P =0.7, wing d12: P =0.08). In 2004, hatch date did not influence any of the growth parameters at d6 or d12. Neither female age nor brood size affected nestling growth in any year of the study.

Table 2.3. Mass (mean ± SD) of nestling tree swallows on the four study sites in 2003 and 2004

		Poplar Creek (PC)[a]	**Demo Pond (DP)**	**Natural Wetlands (NW)**	**Consolidated Tailings (CT)**
Weight Day 6 (g)	2003	13.75 ± 3.18 A n=102	11.64 ± 3.20 A n=48	8.73 ± 2.17 A n=12	9.55 ± 6.42 A n=11
	2004	13.29 ± 2.01 A n=93	12.98 ± 2.08 A n=60	12.34 ± 2.20 A n=26	12.84 ± 2.75 A n=55
Weight Day 12 (g)	2003	23.61 ± 2.37 A n=40	22.00 ± 2.23 A n=21	21.08 ± 1.53 A n=3	_
	2004	24.08 ± 1.56 A n=84	22.68 ± 1.61 B n=53	22.66 ± 2.42 B n=26	22.95 ± 1.73 B n=55

n= number of nestlings
[a] Reference site
Different letters indicate a statistical difference among sites ($P \leq 0.05$).

In 2003, weight (P=0.2) and wing length (P=0.2) of 6 day-old nestlings were not significantly different among sites (Tables 2.3 and 2.4). Although average measurements suggested smaller sizes on reclaimed wetlands, nestlings tended to hatch later on these sites, and differences were no longer significant after correction to a common hatch date. No d12 growth measurements could be carried out on CT because all nestlings died before that age. There was no significant difference in weight and wing length between twelve-day-old chicks from NW, DP and PC (weight: P = 0.9 and wing: P=1.0).

Table 2.4. Wing length (mean ± SD) of nestling tree swallows on the four study sites in 2003 and 2004

		Poplar Creek (PC)[a]	Demo Pond (DP)	Natural Wetlands (NW)	Consolidated Tailings Wetlands (CT)
Wing Day 6 (cm)	2003	2.1 ± 0.5 A n=102	1.8 ± 0.4 A n=48	1.5 ± 0.3 A n=12	1.6 ± 0.8 A n=11
	2004	1.9 ± 0.3 A n=93	1.9 ± 0.3 A n=60	1.7 ± 0.2 A n= 26	1.8 ± 0.3 A n=55
Wing Day 12 (cm)	2003	4.8 ± 0.8 A n=41	4.5 ± 0.6 A n=21	4.6 ± 0.4 A n=3	_
	2004	5.0 ± 0.4 A n=84	4.9 ± 0.3 A n=53	4.8 ± 0.5 A n=26	4.8 ± 0.5 A n=55

n= number of nestlings
[a] Reference site
Different letters indicate a statistical difference among sites ($P \leq 0.05$).

In 2004, there were no significant differences in the body mass and wing length of six-day-old nestlings between reclaimed wetlands and the reference site (weight: P =0.8; wing: P =0.3) (Tables 2.3 and 2.4). Wing length for chicks from reclaimed wetlands was similar to controls at d12 (P = 0.4). However, d12 nestlings from reclaimed wetlands were significantly lighter compared to controls. Chicks on CT (P = 0.03) weighed 1.1g less than those on PC, and those on NW (P = 0.03) and DP (P =0.01) weighted 1.4 g less than nestlings on PC.

2.4.3. Nestling Mortality and Meteorological Conditions

Poor reproductive output in 2003 (section 2.4.1) was related to a weather-related episode of widespread mortality among nestlings. Within a week, a total of 134 nestlings were found dead in their nests during routine monitoring. Gross and histopathological examination was performed on 40 freshly dead nestlings. There were no lesions other than a very mild lymphoid depletion of the bursa of Fabricius in eight nestlings (20%), which was likely stress-related. The die-off occurred between June 22nd and July 2nd and coincided with an abrupt temperature plunge synchronized with heavy and persistent rain. Fig. 2.1 shows nestling mortality in relation to temperature and rainfall variations over time. In 2003, nestling mortality drastically increased just after a 15.8°C temperature drop (from 23.7 °C on June 18th to 7.9 °C June 22nd) which coincided with a peak in rainfall (Fig. 2.1.1). In 2004, in which no mass mortality of nestlings occurred, temperatures oscillated up and down but there was no sudden and sustained drop, and rainfall was minimal (Fig. 2.1.2).

Table 2.5 summarizes meteorological data measured at Syncrude weather station in 2003 and 2004 during the nestling rearing period. Despite the variations in daily patterns illustrated in fig. 2.1, average temperatures over the breeding season were not significantly different among years (P =0.3). There were however striking differences in rainfall among years (Table 2.6). Daily rainfall was about ten times higher in 2003 than in 2004 (P =0.01).

Fig. 2.1.1

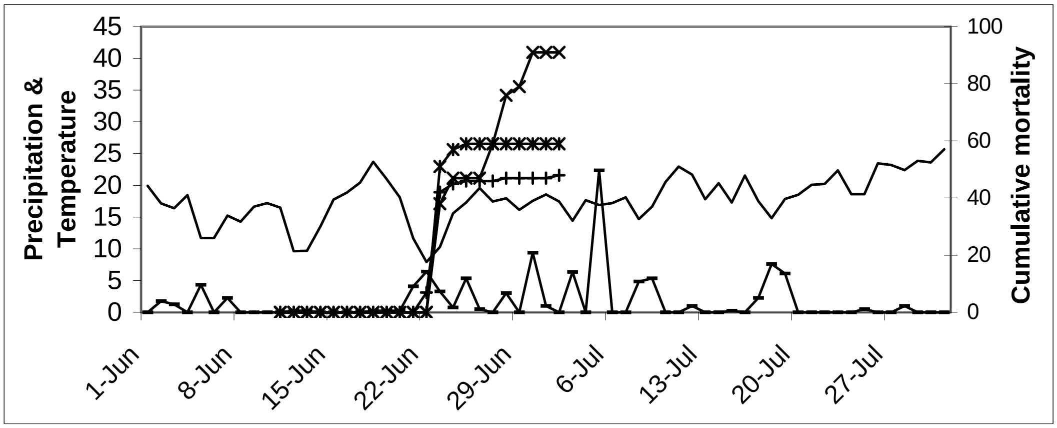

Fig. 2.1.2

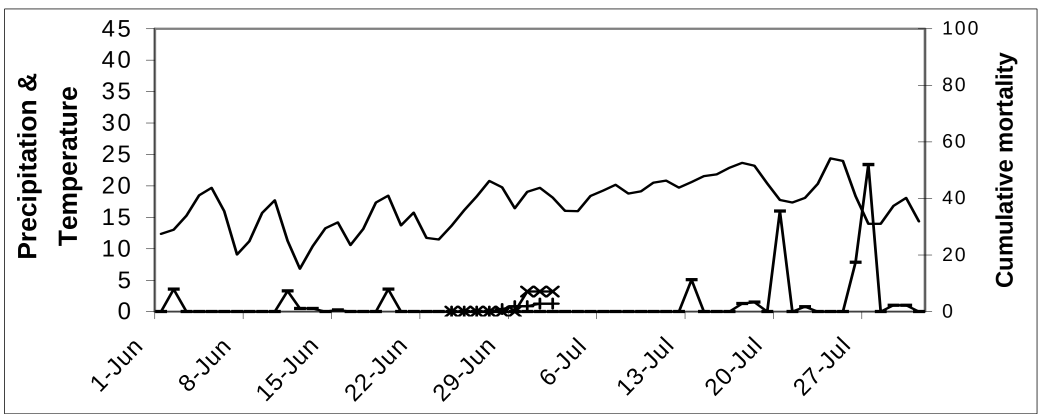

Fig.2.1 Cumulative mortality (%) of nestling tree swallows in 2003 and 2004 in relation to ambient temperature and rainfall

In 2003, mortality was high and was linked to an episode of harsh weather (Fig. 2.1.2), while mortality was low in 2004, as weather was less challenging (Fig.2.1.2). The left axis shows the temperature in Celsius (°C) and the average precipitation in millimeters (mm), while the right axis shows cumulative mortality (%). On the graph, lines without markers represent the temperature and lines with "–"markers represent precipitation. Lines with "X" markers represent cumulative mortality on Suncor sites, Natural Wetlands (NW) and Consolidated Tailings wetlands (CT). Lines with "*" markers represent cumulative mortality on Demo Pond (DP, Syncrude) and lines with "+" markers represent cumulative mortality on Poplar creek (PC, reference site).

Table 2.5. Meteorological data (mean ± SD) measured at a weather station centrally located relative to the study sites[1], from June 6th to July 11th 2003 and 2004

	2003	**2004**
Mean daily temperature (°C)	16.4 ± 3.6 A	15.8 ± 3.7 A
Min-Max daily temperature (°C)	7.9- 23.7	6.8- 20.8
Mean daily precipitation (mm)	2.12 ± 4.25 A	0.23 ± 0.80 B
Total precipitation (mm)	74.0	8.1
Number of days with precipitation	19	5

Different letters indicate a statistical difference among years ($P \leq 0.05$)

[1] Data from Syncrude's 30-Dump weather station

Table 2.6 contains descriptive mortality rates for each site in 2003, which were calculated as the total number nestlings found dead on a site, divided by the number of nestlings alive on that site prior to the harsh weather. Nestling mortality occurred on all study sites, but the death toll was particularly high on the NW (89.3%) and CT (100%) wetlands. Site was significantly associated with nestling mortality (P=0.045) (CT excluded because of the small sample size). Even though hatch date was not significantly (P=0.4) associated with mortality, it was included in the model as a covariate because it was very likely a confounding factor, as nestlings on the sites with the highest mortality rates tended to hatch later than on other sites (Table 2.7).

Table 2.6. Descriptive mortality rates (%) of nestling tree swallows on the four study sites in 2003 and 2004

	Mortality Rate[b] (%)	
	2003	2004
Poplar Creek (PC)[a]	48.0 % (73/152)	2.8 % (3/106)
Demo Pond (DP)	58.8 % (30/51)	0 % (0/52)
Natural Wetlands (NW)	89.3 % (25/28)	3.6 % (1/28)
Consolidated Tailings (CT)	100 % (6/6)	0 % (0/54)

[a] Reference site
[b] Mortality rate = total number of dead chicks/total number of chicks alive prior to the die-off (nestlings victims of predators excluded).

Table 2.7 Mean hatch date of nestling tree swallows on the four study sites in 2003

	Hatch date	**SD**	**n**
Poplar Creek (PC)[a]	June 16th	2.4	152
Demo Pond (DP)	June 16th	2.9	51
Natural Wetlands (NW)	June 19th	1.9	28
Consolidated Tailings Wetlands (CT)	June 17th	.000	6

[a] Reference site
SD= standard deviation, n=number of nestlings

When hatch date (as a covariate) and site (as a main effect) were tested simultaneously, both were highly significant (hatch date: P=0.01; site: P=0.005). The interaction between site and hatch date was close enough to significance (P=0.06) for its biological importance to be noted, as shown in fig. 2.2. Fig. 2.2 illustrates the predicted probability of dying for nestlings of different hatch dates (CT excluded). On NW, nestlings had very high probabilities of dying regardless of their age (hatch day), while on PC and DP the association between hatch date and probability of dying was negative. This indicated that on these two sites, younger nestlings had better chances of surviving compared to older ones, particularly on DP (the slope was steeper on DP).

Only four nestlings died in 2004. There was no association between site and nestling fate (P =0.5).

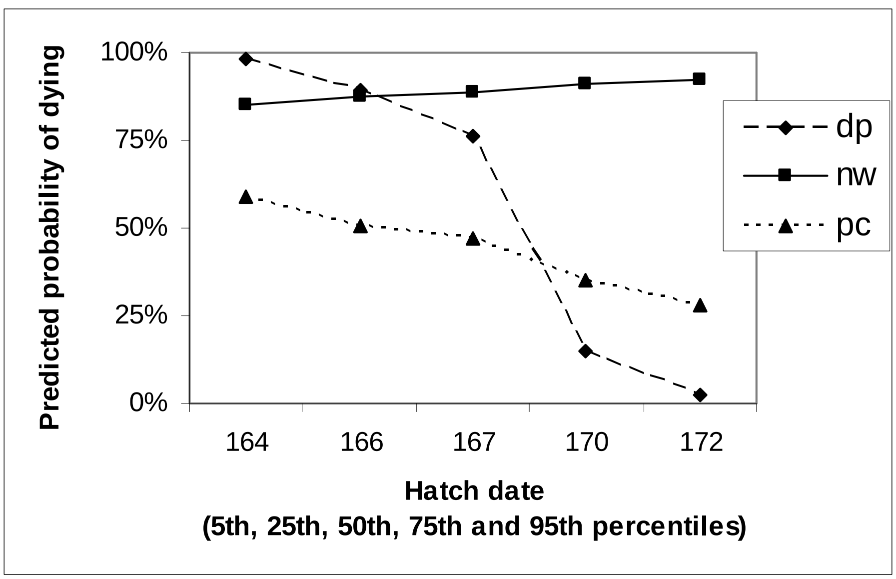

Fig. 2.2. Predicted probability of dying of nestling tree swallows.

DP=Demo Pond (Syncrude), NW=Natural Wetlands (Suncor), PC=Poplar Creek (reference site). Hatch date are in Julian days, where 164=June 13th, 165=June 14th, and so on.

2.4.4 EROD Activity

Nestlings from CT had the highest ethoxyresorufin-*o*-deethylase activity, closely followed by those from NW (Table 2.8). In chicks from CT, EROD was 2 times higher than in those from the reference site (P =0.02), and in those from NW, it was 1.9 times higher than in nestlings from PC. Differences between NW and PC approached statistical significance (P=0.07). Enzymatic activity in nestlings from DP was similar to that in nestlings from the PC site (P= 0.8).

Table 2.8. Ethoxyresorufin-o-deethylase (EROD) activity (mean ± SD) in the liver of nestling tree swallows on the four study sites in 2004

	EROD Activity	**n**
Poplar Creek (PC)[a]	38.0 ± 23.2 A	16
Demo Pond (DP)	45.7 ± 36.8 AB	16
Natural Wetlands (NW)	72.3 ± 27.7 AB	16
Consolidated Tailings Wetlands (CT)	74.2 ± 44.3 B	20

[a] Reference site
n= number of nestlings
Different letters indicate a significant difference among sites ($P \leq 0.05$).

2.5. DISCUSSION

2.5.1. Tree Swallow Reproduction

Reproductive output was very low on reclaimed wetlands in the cold and rainy summer of 2003, especially on CT and NW. In contrast, reproductive success was not different among sites in 2004. These results strongly suggest that the harsh weather contributed to the low reproductive success. Fledging success and nest success on OSPM sites were much lower than on the reference site and were among the lowest ever reported in a passerine species used as environmental sentinel, suggesting that inclement weather alone was not responsible for the poor reproduction. In most ecotoxicology studies, which were conducted under moderate weather conditions, the reproduction of birds on sites affected by industrial processes has not been as compromised. Fledging success of tree swallows exposed to PCBs ranged from 51% to 74% (Bishop et al., 1999) and nest success varied between 50% and 80% (McCarty and Secord, 1999). Nest success ranged from 75% to 77% in great tits (*Parus major*) breeding in the vicinity of a metal smelter (Janssens et al., 2003). The findings of this study are of concern because of the large scale of mining operations (and eventually mine reclamation). If reproductive performance of birds on all Oil Sands reclaimed areas were as impaired as what we documented on our study sites in 2003, low recruitment of young into the breeding population would be inevitable, and if the trend was consistent for several years there could be negative consequences on population dynamics. However, since the poor reproductive success was associated with sustained inclement weather during the nestling rearing period, which is relatively infrequent, it is unlikely that such low productivity would occur every year.

Reproductive success in 2004 was not different between contaminated wetlands and the reference site in 2004. It was comparable to what has been reported for tree swallow populations on non-contaminated sites (Roberston et al., 1992) and to what had been documented on these same study sites in previous years (Smits et al., 2000). Broods were significantly smaller on DP than on PC in 2004, but this was linked to three complete nest failures (nests where no eggs hatched), which could not be clearly related with contaminant exposure. We frequently observed American kestrels (*Falco sparverius*) hunting on DP, trying to reach inside the nest boxes. It is likely that kestrels caught incubating females or disturbed them enough to cause nest abandonment.

2.5.2. Nestling Growth

There were no differences in nestling size among sites in 2003. It is possible that the small sample size may have masked any differences in nestling size. Because most nestlings died before d12, only three nestlings (from one nest) could be measured on NW, and none on CT. Moreover, mortality may have masked differences in growth rates. The smallest nestlings of a brood are the most likely to die during episodes of food shortage (Bryant 1978). Since survival rates were very low on NW, smaller nestlings would likely have died, skewing the mean toward higher weights. Differences in growth rates between reclaimed wetlands and the control site might have been detected if mortality rates had been similar on all study sites.

In 2004, chicks on reclaimed wetlands weighted 4 to 5 % less than those on the reference site. These results contrast with those from previous ecotoxicology studies with tree swallows, where growth rates of nestlings on industrial sites have been unaffected (Bishop et al., 2000b, Harris and Elliott, 2000, McCarty and Secord, 1999). Wayland et al. (1998) even reported higher growth rates downstream from pulp mills than upstream from them. Food availability is one of the main factors determining chick's growth (McCarty and Winkler, 1999). It was hypothesized in the above studies that nestlings grew normally because insect abundance was not affected (or was increased) by environmental contamination. The situation could be different on the Oil Sands. Laboratory experiments showed that exposure to OSPW can impair the growth and survival of benthic invertebrates (Whelly, 1999), and Demo Pond was unable to support a viable population of aquatic invertebrates in 1997-1998 (Whelly et al., 1998). Nestlings could have been smaller on this site because of reduced food availability. Conversely, benthic invertebrate biomass has generally been higher in NW than in neighboring references wetlands (Bendell-Young et al., 2000, Ganshorn, 2002), but nestlings reared on this site have been consistently smaller than on other sites since 1998 (Smits et al., 2000). This suggests that factors other than insect biomass, including contaminant transfer through the food webs, may be responsible for compromised tree swallow growth rates on OSPM-sites. For instance, exposure to NAs resulted to decreased weight gain in laboratory mammals (Rogers, 2003). Another factor to consider is the benthic species composition. Species diversity has been found lower in OSPM wetlands than in those unaffected by mining (Bendell-Young et al., 2000, Leonhardt, 2003). On a site with very low invertebrate diversity, food availability to swallows may be compromised because most insects would emerge around the same period. Food supply would be high during and shortly after insect emergence, but would be much less abundant during the rest of the nestling rearing period. It would be useful to measure aerial insect abundance and species diversity over the summer to detangle the respective effects of contaminant exposure and food availability on nestling growth.

Regardless of the cause, nestlings from reclaimed wetlands may suffer lower post-fledging survival as a result of their smaller size. It has been repeatedly shown that post-fledging survival is strongly correlated with body mass at fledging (Naef-Daenzer et al., 2001, Ringsby et al., 1998, Tinbergen and Boerlijst, 1990).

2.5.3. Nestling Mortality and Meteorological Conditions

Episodes of inclement weather often result in bird mortality. Aerial insectivores such as swallows are particularly susceptible to abrupt variations in environmental conditions, as rain, wind and cold temperatures may ground the insects and make them unavailable for foraging (McCarty and Winkler, 1999). Moreover, cold temperatures exert negative effects directly on nestlings. Young altricial chicks are ectothermic and require sustained parental brooding to develop normally. Older chicks are homeothermic, but the energetic costs of thermoregulation may surpass energetic reserves, especially when foraging is compromised, and cause great metabolic stress (Visser, 1998). The limiting effects of cold climate on nestling development and survival might be particularly important in this study. Indeed, our sites were located farther north than in most previous research with tree swallows, which was conducted in northern United-States or southern Canada.

Nevertheless, mortality rates on the reference site PC (48%) and the 10-year old reclaimed site DP (58%) were comparable to those reported for a similarly weather-related die-off of purple martins in Pennsylvania, where 58% of the nestlings died (Hill and Chambers, 1992). Mortality rates on younger reclaimed sites CT (100%) and NW (89%) are however extreme compared to what has been documented for birds unchallenged by contaminants. Although large numbers of adults occasionally perish following spring cold spells at their arrival on breeding grounds (Brown and Bomberger Brown, 1998, DuBowy and Moore, 1985), such widespread mortality of nestlings is unusual and has only been documented once in tree swallows (Shutler and Clark, 2003).

On sites with low levels of contaminants such as DP and PC, hatch date was negatively associated with the expected probability of dying (Fig. 2.2). Since the harsh weather started roughly at the same time on all sites (all sites are located within a 10 km radius from the weather station) and no nestlings hatched during or after the harsh weather, hatch date on that graph is representative of nestling age at the onset of harsh weather. That is, nestlings that hatched later (higher julian hatch day) were younger at the onset of harsh weather. Thus our data indicate that on DP and PC, younger nestlings (later hatch date) tended to survive the harsh weather more successfully (had a lower probability of dying) than older nestlings (lower julian hatch day). This could be because younger nestlings have relatively lower energy requirements (Winkler and Alder, 1996) and because they are able to use their poikilothermy as a survival strategy, entering a state of torpor with low metabolic activity when subjected to low temperatures (Visser, 1998). However, on NW, where levels of PAHs and NAs were high, all nestlings had high probabilities of dying regardless of their age, suggesting that they were unable to withstand additional stressors such as cold weather.

Localized storms or precipitation events could have hit CT and NW more heavily than the others, resulting in higher mortality rates on these two sites. However, this would be unlikely, because all the study sites are within a 12km radius from Syncrude weather station. There were no significant differences in rainfall and temperature between Suncor's and Syncrude meteorological stations (data not shown). However, meteorological data for the control site were unavailable. It would have been necessary to record data at each of the sites to completely discount this possibility.

Nonetheless, site-specific environmental factors, such as the exposure of the nest boxes to wind and rain, could have affected nestling survival. Paradoxically, boxes on the reference site (PC) were the least protected from the elements, whereas those on Suncor were the most sheltered. Although PC is bordered by mixed forest composed of white spruce (*Picea glauca*), balsam poplar (*Populus balsamifera*) and trembling aspen (*Populus tremuloides*), nest boxes are perched on the exposed shore of the 1.25 km^2 reservoir, facing the water. During episodes of inclement weather, strong winds over the lake pushed the rain into entrance holes, which often dampened the feathers lining the nests and the chicks. A similar situation was found on Syncrude, where boxes faced the 5 ha pond and were surrounded by grassy areas devoid of shrubs or trees. Nestlings on PC and DP did not appear very protected from cold ambient temperatures. In contrast, dense shrub communities (*Salix sp., Populus sp., Potentilla fruticosa*) grow around boxes on NW. CT site offers intermediate sheltering, as boxes are partly protected by young (1.7 to 2 m high) white spruces. If chick mortality were correlated with nest exposure, we would have expected it to be the worst on PC and DP, not on CT and NW. This also implies that if the nests on PC were better protected, the differences in survival between this site and Suncor reclaimed wetlands would have been even more dramatic.

Concentrations of NAs and presumably PAHs are highest on CT, closely followed by NW. Exposure to these contaminants could have impaired nestlings' ability to tolerate additional natural stressors, including hypothermia and food shortage. Alternatively, the large energetic demands for thermoregulation could have triggered a decrease in hepatic detoxification, leading to intoxication. Either case could explain why we did not observe any die-off in 2004, when the weather was less challenging. A small number of studies have shown that chemical toxicity can be exacerbated by other stressors. Reduced food intake increased DDE toxicity in ringed doves (Keith and Mitchell, 1993). The synergistic effects of mercury, adverse weather, and food shortage have been reported as a cause of mortality in common loons (Daoust et al., 1998). Fish experimentally exposed to cold water were more susceptible to selenium toxicity (Lemly, 1993). Fish are poikilotherms, so their response to temperature stress might be different from that of an adult bird or an older nestling. However, it could possibly be comparable with that of a young, ectothermic nestling.

The current findings suggest that nestlings reared on OSPM-sites are less able to withstand additional stressors than those on areas unaffected by Oil Sands mining. This raise concern regarding juvenile survival of tree swallows fledged at OSPM sites because altricial birds cope with important challenges shortly after fledging, as they must learn to become nutritionally independent, start to disperse and undertake the winter migration (Kershner et al., 2004). Juveniles that are less fit, such as those that were reared on OSPM sites, may suffer excessive juvenile mortality after leaving their nest. Yet, the period immediately following fledging is rarely monitored in ecotoxicology studies because of logistic difficulties. As Keedwell (2003) pointed out, leaving the nest does not necessarily equal success.

2.5.4 EROD Activity

Cytochrome P450 activity was significantly higher (compared to PC) in nestlings on the most recent reclaimed site CT, which harbored the highest concentrations of NAs and presumably PAHs, while on ten year old Demo Pond, where concentrations of those chemicals were low, EROD was comparable to that of nestlings on the reference site. This was likely linked to the presence of PAHs and NAs in nestlings' diet, which would be expected to vary according to the degree of contamination in water and sediments. Custer at al. (2001) similarly documented EROD induction in tree swallows exposed to PAHs and oral exposure to NAs slightly stimulated EROD in laboratory rats (Rogers, 2003). The EROD data from NW deserves particular attention because differences were very close to statistical significance (P=0.07) and those results have important implications regarding the sustainability of wet landscape reclamation. When EROD was measured in nestlings for this study (in 2004), fresh mining effluents had not been pumped into NW since 2001, i.e., bioremediation had been undergoing for three years. Still, monooxygenase activity in nestlings from NW in 2004 was exactly as stimulated (compared to nestlings from PC) as when it was measured by Smits et al. in 1998, i.e., while fresh tailings were being pumped into NW wetlands. Thus, if we consider that EROD induction on NW was of biological importance, these results indicate that it takes more than three years for microbial activities to detoxify OSPM. Since EROD was not induced on DP (constructed in 1993), we can conclude that bioremediation may take as much as 10 years before contaminant levels in reclaimed wetlands do not induce EROD in tree swallows and possibly other avian species. Our findings contrast with those of MacKinnon and Boerger (1986), who reported that the toxicity of tailings water to aquatic organisms decreased over a one to two years period after being transferred to shallow pits. It is impossible to determine whether birds on reclaimed wetlands suffer from decreased availability of metabolic resources because they have to allocate part of their metabolic resources to detoxification efforts, as EROD could not be measured on nestlings that succumbed to the harsh weather. Nevertheless, since mean mortality rates were highest on sites where mean EROD was highest, our results suggest that monooxygenase induction may be somewhat linked with negative consequences on fitness.

2.5 CONCLUSIONS

During a summer of harsh weather, tree swallow reproductive performance was greatly reduced on reclaimed wetlands containing Oil Sands Process Material compared to control wetlands, and nestlings on reclaimed wetlands had much higher mortality rates than those on the reference site. In the absence of challenging environmental conditions, nestlings from reclaimed wetlands survived to fledging, but were not as large as those on the control site and showed increased hepatic detoxification activity. These results suggest that the implementation of current wet landscape reclamation on a large geographic scale might negatively impact reproductive success of tree swallows and potentially other birds nesting there, but it is difficult to determine to what extent, because the study sites where the tree swallows were the most affected represented early reclamation scenarios, where maturation of tailings in wetlands systems was not yet complete. This study also demonstrates that unless nestlings were subjected to a combination of stressors, they were relatively resilient to chemical exposure. Limiting biomonitoring to the pre-fledgling period might underestimate the consequences of exposure to contaminants, because nestlings are relatively unchallenged until they leave their nest.

CHAPTER 3

Parasitism of Tree Swallows Nestling on OSPM-sites With Larvae of the Bird Blowfly *Protocalliphora* spp.

3.1 ABSTRACT

Oil sands mining companies in Alberta, Canada, are planning reclamation strategies in which wetlands will be used for the bioremediation of water and sediments affected by the oil sand extraction process. Prevalence and intensity of infestation with *Protocalliphora sp* (Diptera: Calliphoridae) were measured in nests of tree swallows (*Tachycineta bicolor*) on wetlands affected by the mining process and compared with those on a reference site. Prevalence of infestation on our sites was among the highest ever reported for a small cavity-nester: all 38 nests examined were infested. Nests on all three OSPM-sites contained on average 60% to 72% more larvae than those on the reference site, and nestlings suffered individual burdens about twice that of control nestlings. For comparable parasitic burdens, nestlings on OSPM-sites suffered greater pathological effects (decreased growth) than those on PC. The high blowfly infestation on OSPM-impacted wetlands may have resulted from different habitat characteristics, the scarcity of blowfly predators on reclaimed wetlands, or increased host susceptibility to parasites. This work emphasizes the value of incorporating parasitology into wildlife toxicology research, in addition to the traditional endpoints.

3.2 INTRODUCTION

Birds are widely used as biological indicators of environmental pollution (Grasman and Fox, 2001, Janssens et al., 2003). Most studies traditionally focus on an evaluation of reproductive performance, nestling growth and biochemical endpoints reflecting detoxification efforts such as hepatic ethoxyresorufin-*o*-deethylase activity (EROD). To the author's knowledge, only one study has investigated the link between exposure to contaminants and ectoparasite infestation in free-ranging birds (Eeva et al., 1994). Larvae of the blowfly *Protocalliphora* spp. (Diptera: Calliphoridae) are obligate hematophagous parasites of altricial birds. They live in nest material and feed intermittently on nestlings' blood until pupation. The infestation of young birds by *Protocalliphora* attracted attention as early as 1845 (Sabrosky et al., 1989). Since then, this complex host-parasite relationship has been the subject of much research, most of which has focused on biological consequences of infestation for the nestling host (Whitworth and Bennett, 1992, Hurtrez-Boussès et al., 1997, O'Brien et al., 2001). However, the ecological factors that regulate *Protocalliphora* populations and that influence the prevalence and intensity of infestations remain largely unknown.

This research was conducted on the oil sands of north eastern Alberta, Canada. The Athabasca deposit reaches approximately 42,000 km^2 and represents one of the world's largest reserves of crude oil (Alberta Department of Energy, 2005). Separation of the heavy crude oil from sand is accomplished by the addition of large volumes of hot water. Once the bitumen has been extracted, residual sand, clay, and process water, along with organic and inorganic contaminants are diverted to vast settling ponds on the mining sites (Mikula et al., 1996). These tailings are accumulating at the rate of $\sim 10^5$ m^3/day (Madill et al., 2001), and since there is currently no discharge of the mining effluents from the mine leases, as much as 1 billion m^3 of oil sands tailings will require detoxification and reclamation upon mine closure (FTFC 1995).

As a result of leaching from the ore and the addition of chemicals during processing, the water used in the extraction of bitumen (Oil Sands Process-Affected

Water, OSPW) becomes contaminated with polycyclic aromatic hydrocarbons (PAHs) and dissolved organic chemicals such as naphthenic acids (Mikula et al., 1996). Process-affected water has been shown to be toxic to plankton communities (Leung et al 2003), fish (van den Heuvel et al., 2000) and amphibians (Pollet and Young 2000). Reclamation of the mining areas has included the creation of artificial water bodies (wet landscape reclamation) to receive aging tailings, which are capped with a layer of fresh water (Gulley and MacKinnon 1993). Most of the acute toxicity to aquatic organisms should be mitigated by natural processes such as microbial biodegradation, but long-term ecological impacts may still occur (Lai et al., 1996).

To investigate the feasibility, efficiency and sustainability of the wet landscape reclamation strategy, the two largest oil sands mining companies, Syncrude Canada Ltd. and Suncor Energy Inc., have constructed in the past 15-20 years a series of experimental wetlands and test ponds, which were partly filled with mine tailings to mimic future reclamation plans. Tree swallow nesting on these sites are a good species in which to study the transfer of oil sands compounds through food webs, because insects of aquatic origin, whose larvae develop in intimate contact with contaminants present in water and sediments, account for over 80 % of their diet (Smits et al., 2000). In 2004, the prevalence and intensity of *Protocalliphora* infestation on OSPM wetlands were compared with that of a reference site. The associations between parasitic load and growth and survival of nestlings, and the impact of OSPM exposure on parasitism were investigated.

3.3 METHODS

3.3.1 Study Sites

The study was conducted on one reference site and three wetlands containing OSPM located on Suncor's and Syncrude's leases (57º 00' N, 111º 30 E), from late May to mid July of 2004. Please consult Chapter 1 for a detailed description of Poplar

Creek reference site (PC), Consolidated Tailings (CT) wetlands and Natural Wetlands (NW) on Suncor, as well as Demo Pond (DP) on Syncrude (Fig.1.4).

3.3.2 Monitoring of Tree Swallow Activity

Old nest material was cleared from all boxes before the arrival of the birds in the spring. Tree swallow activity was monitored from May 19^{th} to July 15^{th} 2004. Nest boxes were inspected daily during the laying period. Completed clutches were left undisturbed until hatching of the nestlings. Each nestling was uniquely identified with different color combinations on the claws. Nestling weight and wing length were measured on day 6 and day 12 (hatch day = day 1). Nestling mass was measured to the nearest 0.05g using a Pesola spring balance and wing length (carpus to end of longest primary feather) was measured to the nearest 0.1cm. Although tree swallow nestlings normally leave the nest 18 to 21 days post-hatch, those that survived to day 14 were considered “fledged” for the purpose of the study and then left undisturbed to avoid premature fledging. Nestlings lost to predators were excluded from the analysis of survival.

3.3.3 Nest and Nestling Examination

A complete parasite examination was carried out on all nestlings at day 12. This included systematic inspection of the chicks to record lesions induced by feeding larvae (pinpoint reddish-brown scabs on the skin or at the base of emergent feather shafts). Larvae detaching from the chicks during handling were returned to the nest. Within seven days of the birds’ fledging, nest material was collected and placed individually into sealed plastic bags. Nests where no eggs hatched or where all the chicks were lost to predators before reaching 12 days were not collected. Bags were stored at room temperature until shipping to Dr Withworth, an entomologist specializing in bird blowflies. Nest examination was conducted using identification keys described in Whitworth, 2002. Bag content was emptied on a large sheet of white paper and nest material was dissected to count pupae and empty puparia (outer shell left after the adult fly emerged). In this paper, we report “total puparia”, because the nests contained mostly empty puparia at the time of examination. Mean nestling load reported here is based upon total puparia in a given nest divided by the number of nestlings occupying this nest (total puparia/brood size).

3.3.4 Statistical Analysis

Data from nestlings of a same brood exhibit some degree of correlation. Unlike classical statistical tests, which rely on the assumption that all observations are independent, multilevel models are designed to analyze hierarchical data (clustered data). For instance, individuals clustered within households, clustered within different geographical areas can be surveyed, using statistical equations that properly include all the appropriate dependencies (Hox, 2002, Dohoo et al., 2003). This approach has recently been used in ecological research with nestling birds (Simon et al., 2004). In this study, we used linear mixed models (one technique of multilevel analysis) to detect associations between SITE (DP, PC, NW, CT) and continuous outcomes (e.g. nestling weight, wing length), including a random intercept for “nest” in each model to account for the lack of independence among chicks from the same brood (PROC MIXED, SAS for Windows version 8.02, SAS Institute Inc., Cary, NC.). The associations between SITE and discrete outcomes were similarly analyzed using generalized estimating equations (PROC GENMOD, SAS for Windows version 8.02). A Poisson distribution with log link function was used for count outcomes (e.g., puparia, lesions). A binomial distribution with logit link function (logistic regression) was used to determine if parasitism affected survival to fledging. Because infestation can increase over the breeding season and larger broods may attract more blowflies (Hurtrez-Boussès et al., 1999, Wesolowski, 2001), hatch date and brood size were considered as potential covariates and were included in the models when $P \leq 0.05$ (or when inclusion of the factor in the model changed the P value by more than 10%).

3.4 RESULTS

3.4.1 Patterns of *Protocalliphora* Infestation

In 2004, 246 chicks were inspected for larval feeding lesions (scabs), and 38 nests were collected for examination. Of the nests examined, 100% were infested with *Protocalliphora sp.* The most common species was *Protocalliphora sialia*; *P. bennetti* was present in lower numbers. One nest had two *P. braueri* (*Trypocalliphora braueri*) pupae. Mixed infestations (with two species or more) occurred in 59 % of the nests. Nest burden varied from 9 puparia (on PC) to 125 puparia (on NW), and mean nestling load (puparia/brood size) ranged from 2 to 25 (Table 3.1). Hatch date was not associated with the number of puparia in a nest (P=0.2) or with nestling burden (P= 0.2), but nestlings hatched later in the season exhibited more lesions (P= 0.002). The number of lesions on a nestling (Table 3.2) was not correlated with the total number of puparia in the nest where it was reared (P= 0.1), or with its mean nestling load (P = 0.07). Brood size was not associated with any measure of infestation.

Table 3.1. Intensity (mean ± SD) of *Protocalliphora* infestation in nests of tree swallows on the four study sites in 2004

		Poplar Creek (PC)[a]	**Demo Pond (DP)**	**Natural Wetlands (NW)**	**Consolidated Tailings (CT)**
Nest burden (total puparia)	Mean	44.1 ± 24.2 A	75.0 ± 19.2 B	76.0 ± 38.0 B	70.6 ± 22.0 B
	Range	9-75	35-95	30-125	40-100
Nestling burden (puparia/ brood size)	Mean	6.8 ± 3.5 A	14.7 ± 3.4 B	15.1 ± 7.9 B	11.5 ± 2.9 B
	Range	2-12	9-19	7-25	6-14
n		17	7	5	9

n= number of nests
[a] Reference site
Different letters indicate a statistically significant difference ($P \leq 0.05$)

3.4.2 *Protocalliphora* Infestation and Exposure to OSPM

Protocalliphora infestation was greater in nests on OSPM-impacted wetlands compared to those in nests from the reference site (Fig.3.1). Compared with PC reference site, nests from DP (P =0.0007), NW (P =0.02) and CT (P =0.004) harbored 1.6 to 1.7 times more puparia. Mean nestling burden of parasites was 2.2 times heavier on DP (P < 0.0001) and NW (P <0.0001) than on PC, while it was 1.7 times higher on CT than on PC (P =0.004) (Table 3.1). Chicks on reclaimed wetlands had more skin lesions than controls, but they also tended to hatch later (data not shown), and since the number of skin lesions increased as the season progressed, site differences were no longer significant after inclusion of hatch date as a covariate in the model (P = 0.08).

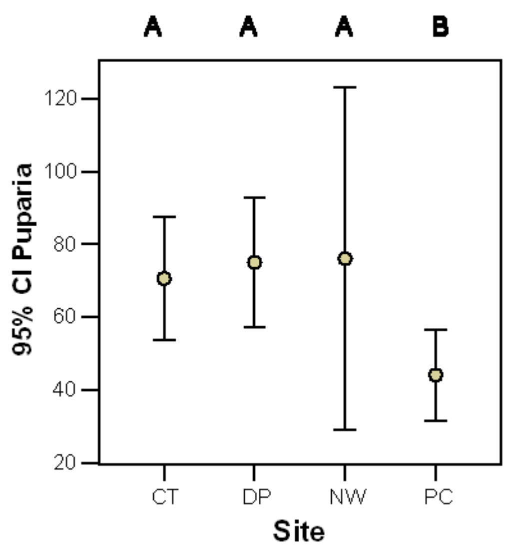

Fig. 3.1. Parasitic burden of nests on the different study sites. Different letters above the graph indicate statistically significant differences ($P \leq 0.05$).

Table 3.2. Skin lesions (mean ± SD) left by feeding blowfly larvae on tree swallow nestlings on the four study sites in 2004

	Poplar Creek (PC)[a]	**Demo Pond (DP)**	**Natural Wetlands (NW)**	**Consolidated Tailings (CT)**
Number of lesions	4.2 ± 3.4 A	8.5 ± 7.4 A	8.7 ± 7.9 A	9.9 ± 8.5 A
Range	0-19	0-38	0-30	0-29
n	112	53	26	55

[a] Reference site
n= number of nestlings
Different superscripts letters indicate a statistical difference (P <0.05)

3.4.3 Nestling Growth

Nestlings on reclaimed wetlands were significantly lighter than those on the reference site. On CT, they weighed 1.1 ± 1.7 g less than those on PC (P= 0.03). On NW (P = 0.03) and DP (P =0.01) they weighed 1.4 ± 2.4 g and 1.4 ± 1.6 g less, respectively, than those on PC (Chapter 2, Table 2.3). Nestling body mass decreased as numbers of puparia in nests increased (P = 0.0003) and as mean individual burden increased (P = 0.0004). Wing length was not associated with nest loading (P =0.2), mean nestling burden (P =0.7) or number of skin lesions (P =0.5). The number of skin lesions was not correlated with nestling weight (P = 0.6).

Fig. 3.2 illustrates the effect of nest parasitic burden on nestling weight. On the three reclaimed sites, nestling weight decreased as nest burden increased, while weight seemed relatively unaffected by parasitism on PC. When data were analyzed separately for the reference site and the reclaimed wetlands (lumped together), body mass was not associated with nest burden on PC (P = 0.8). However, there was a strong negative correlation between body mass and parasitism on OSPM wetlands (*P*=0.0004). This strongly suggested the presence of a statistical interaction between site and nest burden, therefore, the effects of site and nest burden on nestling weight were compared simultaneously. In spite of the biological evidence, the interaction was not statistically significant (*P*=0.09). This was likely driven by the small sample size on each of the reclaimed sites. When the combined effects of "site status" (reference versus OSPM) and parasitic burden on nestling weight were examined, the interaction between site status and nest burden became significant (*P*=0.01).

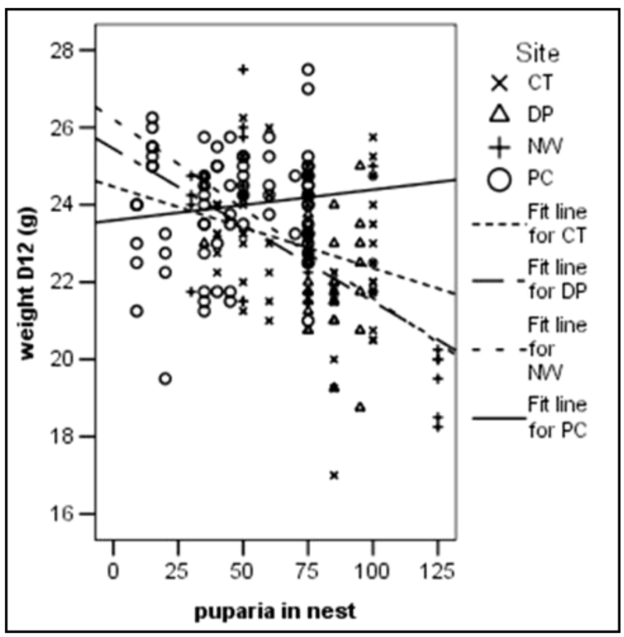

Fig. 3.2. Nestling weight at day 12 in relation to nest parasitic burden. On the three oil sands sites (CT, DP, NW), nestling weight decreases as nest burden increases, while nestling weight is unaffected by parasitism on the reference site (PC). CT=Consolidated Tailings wetlands, DP=Demo Pond, NW=Natural Wetlands, PC=Poplar Creek.

3.4.4 Nestling Survival

Only four nestlings died during the course of the study, and mortality did not differ across sites (P=0.5). Whether a nestling survived to fledging or died was not related to its number of skin lesions (P = 0.2), to the number of puparia in the nest it was reared (P = 0.5) or to the mean nestling burden of its brood (P =0.3).

3.5 DISCUSSION

3.5.1 Patterns of Infestation

Total nest burden (puparia/nest) and nestling load (puparia/brood size) on our study sites were comparable to what has been previously reported for tree swallows (Rogers et al., 1991, Bennett and Whitworth, 1992, Roby et al., 1992, Thomas and Shutler, 2001, Dawson et al., 2005). However, to the author's knowledge, we found on our study sites the highest prevalence of infestation (100%) for tree swallows, and possibly for other species of small cavity nesters. The studies mentioned above have reported prevalence ranging from 54% to 89%. In other species of small cavity-nesters, the highest prevalence (96.5%) has been documented in one population of Corsican Blue Tits (Hurtrez-Boussès et al., 1999).

It is unclear why prevalence of bird blowfly infestation was so high on our study sites. Bennett and Whitworth (1992) showed that birds exhibiting high nest fidelity, returning to the same sites year after year, tend to be heavily infested with *Protocalliphora* sp. Tree swallows have occupied nest boxes on our study sites for a maximum of 10 years, and this could have allowed large and stable populations of *Protocalliphora* to develop. Yet, well-established colonies have been monitored in other studies (Rogers et al., 1991), and prevalence did not surpass 77%. Alternatively, if other suitable host species nesting in natural cavities were scarce in the area because of the cumulative disturbance associated with mining operations, female flies would be more likely to lay eggs in our nest boxes.

Mixed infestations on our sites were very common. Tree swallow nests in northern British-Columbia also had high rates of mixed infestations; 66% of the nests harbored two or more species (Dawson et al., 2005). In contrast, Bennett and Whitworth (1992) found only 12.5 % of the nests from the Great Lakes region of Ontario and 6.5% of those from Utah to have mixed infestations.

3.5.2 *Protocalliphora* Abundance: Habitat Characteristics

Nests and nestlings on all three OSPM-impacted sites exhibited much heavier infestation than those on the reference site. Perhaps habitat characteristics specific to each study site influenced blowfly abundance. Bennett (1957) first suggested that blowflies may be deterred by exposure to wind and rain, and Heeb et al. (2000) experimentally showed that humid nests were less likely to be infested with *Protocalliphora* than dry nests. Recently, Dawson et al. (2005) experimentally manipulated temperatures in tree swallow nests infested with blowfly larvae and found that larval densities decreased at both high and low temperatures, with the largest numbers of larvae being found at 25°C. Nest boxes on the reference site (PC) are perched facing the water on a dyke which forms the shore of a 125 Ha reservoir. In early spring and during episodes of inclement weather, strong winds over the lake pushed the rain into entrance holes, which often dampened the nests and the chicks. Increased humidity and lower temperatures could have explained the smaller parasitic loads on this site, except that the same effect was not evident on DP, although boxes faced the 5 ha pond and there was no sheltering vegetation at that site. Yet, nestling burden on DP was more than twice that of PC.

High nest density has been shown to increase the rate and intensity of infestation with *Protocalliphora* (Brown and Brown, 1986, Shields and Crook, 1987). Nest density was no greater on OSPM sites than on the control site. In fact, PC has the highest number of boxes and the highest nest density, but it was the least infested. Whitworth (1976) found that new nest boxes are rarely as infested as older ones. This trend was not verified on our study sites. Nests from the oldest colony (NW, 1995) were as heavily infested as those from the newest colony (CT, 2003). Nest boxes were erected on both DP and PC in 1997, but infestation was much heavier on DP than on PC.

Female flies will lay their eggs in the nests of most cavity-nesters, and the availability of natural cavities may be a factor limiting blowfly populations. Consequently, bird blowflies may become more abundant in areas where numerous natural cavities are available. Two of our sites (PC and NW) were bordered by mixed forest dominated by white spruce (*Picea glauca)* and balsam poplar (*Populus balsamifera*), where natural cavities would presumably be available. In contrast, CT wetlands are newly reclaimed and vegetation is still in early successional stages. Nest boxes are surrounded by young white spruce (1.5 m to 2 m high), but there are no old-growth trees in the vicinity. DP is devoid of shrubs or trees and grassy fields surround the nest boxes. If blowlfy abundance depended on the availability of natural cavities, nests from NW and PC would have been the most infested, while those from DP and CT would have been the least.

It has been shown that the volume and quality of nest material are limiting factors in the survival of *Protocalliphora* larvae, possibly because of their need for absorbent material to isolate them from their excrements which are toxic (Whitworth, 1976). Because recent research found no correlations between nest volume and *Protocalliphora* abundance (Rendell and Verbeek, 1996b, Hurtrez-Boussès et al., 1999), nest material was not quantified in this study. Nevertheless, an objective measure of nest volume should be included in future research.

Jewel wasps, genus *Nasonia* (Chalcidoidea: Pteromalidae), are small (2-3 mm) parasitoid Hymenoptera that oviposit in the pupae of bird blowflies and kill them (Peters and Abraham 2004). It has been hypothesized that those parasitic wasps could have a limiting effect on blowfly populations (Davis et al., 1994). There may be a possibility that industrial activities on the oil sands would disturb the equilibrium between *Nasonia*, *Protocalliphora* and avian hosts, but more research is needed to validate this hypothesis. Little is known about the distribution of jewel wasps in Canada, and the oil sands might be too far north for them to thrive.

3.5.3 *Protocalliphora* Abundance: Host Characteristics

Empirical studies repeatedly demonstrate that the distribution of parasites in their host population is highly aggregated, i.e., a minority of individuals harbors the majority of parasites (Grenfell et al., 1995, Boag et al., 2001, Krasnov et al., 2005). It has been suggested that parasites may aggregate on hosts of lower resistance to increase their chances of success (Galvani, 2003). Interestingly, Simon et al. (2003) recently showed that *Protocalliphora* larvae aggregate on the smallest chicks of a brood, which tend to be less immunocompetent than their siblings (Christe et al., 1998). The immune responses of the host exert a critical role against ectoparasites. Proteins present in saliva of hematophagous arthropods are foreign antigens, which elicit potent localized immune responses, mediated by Langerhans cells in the skin, T and B lymphocytes, IgG and IgE antibodies, cytokines and inflammatory cells (Wikel and Alarcon-Chaidez, 2001). In the immunologically competent host, the reaction to being bitten is rapid and initiates degranulation of inflammatory cells which causes histamine release (Wakelin 1996). This can impair parasite engorgement, damage its midgut cells and may provoke detachment from the host (Pruett, 1999). Ectoparasites feeding on resistant hosts show stunted growth (Bowles et al., 1996), inhibited molting (Wikel and Bergman, 1997) and decreased fecundity (Walker et al., 2003).

Several environmental contaminants are known to affect the immune system of wildlife (reviewed by Fairbrother et al., 2004), and a small number of studies have demonstrated relationships between pollution and parasitism. The number of nematodes in arctic breeding glaucous gulls increased with tissue-organochlorine levels (Sagerup et al., 2000), and it was later demonstrated that humoral immunity was impaired by organochlorines in this gull population (Bustnes et al., 2004). Prevalence of infection with a parasitic nematode increased in frogs experimentally exposed to pesticides (Christin et al., 2003), and residual numbers of nematodes were positively correlated with residual mercury concentrations in common eiders collected in the Canadian Arctic (Wayland et al., 2001). Similar conclusions were obtained using laboratory rodents (Luebke et al., 1994; Boroskva et al., 1995). Few studies investigated the association between ectoparasites and contaminant exposure. Eeva et al. (1994) found no changes in ectoparasite burden of birds nesting along a gradient of air pollution, but Moles and Wade (2001) documented an increased prevalence of gill ectoparasites in fish exposed to high levels of PAHs.

Fish living in wetlands affected by oil sands tailings have decreased numbers of white blood cells (Bendell-Young et al., 2000). Chemicals present in OPSM may be similarly affecting the immune function of nestlings. Consequently, parasitic larvae feeding on chicks with poor immune responses would have greater survival and fecundity rates than those developing on chicks with normal immune defenses. If this trend persisted for several years, a large *Protocalliphora* population would eventually develop on reclaimed wetlands.

Nevertheless, it is premature to conclude that increased host susceptibility was responsible for severe blowfly parasitism on reclaimed wetlands, as immune function of nestlings was not evaluated in this project because of logistical constraints. Our data suggest that other ecological factors are involved, because there was not a clear relationship between the intensity of parasitism and the gradient of contaminant exposure. For instance, parasitic load was not heavier on CT, which has the highest concentrations of oil sands related chemicals (NAs and presumably PAHs) than it was on DP, which is the most mature reclaimed site and in which concentrations of those chemicals are much lower.

3.5.4 Nestling Growth and *Protocalliphora* Infestation

Chicks on OSPM wetlands weighed 4 to 5 % less than those from the control site. Chick weight was negatively correlated with increasing parasitic load on reclaimed wetlands, but not on the control site. These findings contrast with those from previous research with tree swallows in which nestling growth was generally not impaired, even for parasitic burdens comparable to what was observed on OSPM sites in this study (Rogers et al., 1991, Roby et al., 1992, Rendell and Verbeek, 1996a, Thomas and Shutler, 2001). Our results suggest that nestlings reared on OSPM wetlands were less able to withstand the energy loss inflicted by parasitism and had to hamper other metabolic processes (such as growth) as a survival strategy. Alternatively, there may be a threshold for parasitic burdens below which no compromise of growth is seen, and average nest burden on PC may have been below this threshold. *Protocalliphora* infestation may cause nestlings from reclaimed wetlands to suffer lower post-fledging survival because of smaller body size. It has been repeatedly shown that post-fledging survival is strongly correlated with body mass at fledging (Tinbergen and Boerlijst, 1990, Ringsby et al., 1998, Naef-Daenzer et al., 2001).

3.5.5 *Protocalliphora* and Nestling Survival

Protocalliphora infestation did not affect nestling survival in this study. Although an association between *Protocalliphora* parasitism and compromised fledging success has been reported in a few studies (Shields and Crook 1987, Puchala, 2004), most of the recent research failed to correlate infestation with decreased survival (reviewed by Simon et al., 2004). However, most of these studies, including ours, were conducted under favorable meteorological conditions. Simon et al., (2004) recently demonstrated that *Protocalliphora* infestation reduces thermogenic and metabolic capacities, suggesting that heavily parasitized nestlings (such as those reared on OSPM sites) may be less able to withstand challenging environmental conditions. More research is needed to investigate the effects of synergistic stressors in wild birds, such as ectoparasitism, hypothermia, food shortage and exposure to toxic chemicals.

3.6 CONCLUSIONS

Around Fort McMurray, northeastern Alberta, Canada, we documented the highest prevalence of nest infestation with *Protocalliphora* spp. ever reported for tree swallows. Nests on wetlands containing water and sediments affected by chemicals related to the oil sands extraction process were 60% to 72% more heavily infested than nests on a control site, and nestlings suffered parasitic burdens approximately twice as heavy than those on the reference site. Nestling growth was negatively affected by parasite load on OSPM sites but not on the reference site. Differences in parasite load between contaminated and reference sites did not appear related to habitat characteristics such as sheltering of the nest boxes from the wind and rain, nest density, or age of the colony. Differences in abundance of blowfly predators or in host susceptibility are factors which could modulate the intensity of parasitism. This work emphasizes the value of incorporating parasitology into wildlife toxicology research, in addition to the traditional endpoints.

CHAPTER 4

Thyroid Function of Tree Swallows Nestlings on OSPM Wetlands

4.1 ABSTRACT

Oil sands mining companies are designing reclamation strategies in which wetlands are used for the bioremediation of mining waste materials. To examine the endocrine disrupting potential of chemicals present in Oil Sands Process Materials (OSPM), we measured thyroid hormone concentrations in plasma and hormone content within the thyroid glands of tree swallow (*Tachycineta bicolor*) nestlings on these experimental wetlands. Compared to nestlings from a reference site, plasma triiodothyronine (T_3) was elevated in nestlings from OSPM sites. Within the thyroid gland, total thyroxine (T_4) content (ng hormone/ pair of glands) as well as concentrations (ng hormone/ mg gland tissue) of T_4 and T_3 were increased in nestlings from OSPM-sites. This is the first study investigating endocrine disruption in birds nesting on sites impacted by oil sands mining. Chemicals such as polycyclic aromatic hydrocarbons (PAHs) or naphthenic acids (NAs) as well as other environmental factors may be altering thyroid function of nestlings reared on reclaimed wetlands. Abnormal stimulation of thyroid function may have negative effects on metabolism, behaviour, feather development and molt, which could compromise post-fledging survival.

4.2 INTRODUCTION

Investigating the effects of toxicants on the endocrine system has generated a wealth of research in the past decade. A wide range of species, from invertebrates (Oetken et al., 2004) to amphibians and mammals (Fox, 2001) have been intensely studied, and several contaminants have been identified as endocrine disruptors. Although research initially focused mainly on sex hormones from the estrogen/androgen system, measuring the effects of contaminants on the hypothalamus-pituitary-thyroid axis is now receiving increasing attention (Rolland, 2000, Brown et al., 2004). In avian species, a number of indicators of thyroid function serve as powerful tools for assessing environmental health (Scanes and McNabb, 2003, Mayne et al., 2005). For instance, the structure of thyroid glands can be examined histologically, and triiodothyronine (T_3) and thyroxine (T_4) can be measured in plasma. These two hormones can also be measured directly within the thyroid glands, which often results in more sensitivity than with assays conducted with plasma (reviewed by McNabb, 2005). The thyroid glands of birds contain primarily T_4, and concentrations of T_4 in plasma exceed those of T_3 by approximately 10 fold (Mc Nabb, 2000). Although less abundant, T_3 is considered more active, primarly because the receptors mediating thyroid hormone action in birds bind to T_3 with much higher affinity than they do to T_4 (McNabb, 2000).

Surprisingly, while many industrial pollutants have been scrutinized to detect endocrine disrupting potential, very little attention has been devoted to chemicals associated with oil sands mining. The Athabasca oil sands of north eastern Alberta, Canada, cover over 42 000 km^2 and represent one of the world's largest reserves of crude oil (Alberta Department of Energy, 2005). Separation of the heavy crude oil from sand is accomplished by the addition of large volumes of hot water. Once the bitumen has been extracted, residual sand, clay, and process water along with organic and inorganic contaminants are diverted to vast settling ponds on the mining sites (Mikula et al., 1996). These tailings are accumulating at the rate of ~10^5 m^3/day (Madill et al., 2001), and since there is currently no discharge of the mining effluents from the mine leases, as much as 1 billion m^3 of oil sands tailings will require detoxification and reclamation upon mine closure (FTFC 1995). As a result of leaching from the ore and the addition of chemicals during processing, the water used in the extraction of bitumen (Oil Sands Process-Affected Water, OSPW) becomes contaminated with polycyclic aromatic hydrocarbons (PAHs) and dissolved organic chemicals such as naphthenic acids (Mikula et al., 1996). Process-affected water has been shown to be toxic to plankton communities (Leung et al 2003), fish (van den Heuvel et al., 2000) and amphibians (Pollet and Young, 2000). Reclamation of mining areas has included the creation of artificial water bodies (wet landscape reclamation) to receive aging tailings, which are then capped with a layer of fresh water (Gulley and MacKinnon, 1993). Most of the acute toxicity to aquatic organisms should be mitigated by natural processes such as microbial biodegradation, but long-term ecological impacts may still occur (Lai et al., 1996).

To investigate the feasibility, efficiency and sustainability of the wet landscape reclamation strategy, the two largest oil sands mining companies, Syncrude Canada Ltd. and Suncor Energy Inc., have constructed in the past 15-20 years a series of experimental wetlands and test ponds, which were partly filled with mine tailings (Oil Sands Process-Materials, or OSPM) to mimic future reclamation sites. To date, endocrine disruption of wildlife on these sites has only been assessed in fish. Research has focused on sex steroid hormones but did not provide an assessment of thyroid function (van den Heuvel et al., 1999b, Tetreault et al., 2003). To the author's knowledge, the effects of OSPM on thyroid function have never been investigated in any class of vertebrates. Thyroid function of wildlife inhabiting oil sands reclaimed sites needs to be assessed, because it may be affected by some components of the OSPM such as PAHs and unrecovered bitumen. It has been shown that birds exposed to crude oil (Harvey et al., 1981, Peakall et al., 1981, Jenssen et al., 1990) and fish exposed to PAHs (Stephens et al., 1997, Singh, 1989) suffered altered thyroid hormone levels.

In this study, triidothyronine (T_3) and thyroxine (T_4) were measured in both the plasma and the thyroid glands of nestling tree swallows (*Tachycineta bicolor*) reared on experimental wetlands containing OSPM. Tree swallows have been widely used as sentinels of environmental health and were recently deemed a "new model organism" (reviewed by Jones, 2003). Swallows are a good species to study the transfer of oil sands compounds through the food webs because insects of aquatic origin, whose larvae develop in intimate contact with contaminants present in water and sediments, account for over 80 % of their diet (Smits et al., 2000).

4.3 METHODS

4.3.1 Study Sites

Tree swallows were collected from one reference site and three experimental reclaimed wetlands (containing OSPM) located on Suncor's and Syncrude's leases (57º 00' N, 111º 30 E), from late May to mid July of 2004. Please consult Chapter 1 for a detailed description of Poplar Creek reference site (PC), Consolidated Tailings (CT) wetlands and Natural Wetlands (NW) on Suncor, as well as Demo Pond (DP) on Syncrude (Chapt. 1, Fig. 1.4).

4.3.2 Tree Swallows

Tree swallow activity was monitored from May 19th to July 15th. Nest boxes were inspected daily during the laying period to determine the date of clutch initiation. Completed clutches were undisturbed until anticipated hatching of the nestlings. At 14 days post-hatch, a subset of randomly selected nestlings (2 chicks per nest, selected by picking numbers in a hat) was collected. Nestlings were anesthetized with halothane inhalation (Halothane B.P., MTC Pharmaceuticals, Cambridge, Ontario, Canada), blood was collected by cardiac puncture, then nestlings were euthanized by cervical dislocation. Once nestlings were euthanized, thyroid glands were excised, trimmed of adipose tissue, weighed to the neared 0.001g and inserted into cryovials. They were frozen in liquid nitrogen (-170 °C) within 10 minutes of euthanasia. Frozen thyroids were sent to Dr Anne McNabb from Virginia Tech for hormone analysis (Blacksburg, VA, USA).

4.3.3 Thyroid Hormone Concentrations in Plasma

Thyroid hormones in plasma were measured with a double antibody radioimmunoassay (RIA), using small sample volumes of 12.5 μL for T_4 and 25 μL for T_3, as described by Wilson and McNabb (1997). Primary antibodies for both assays were obtained from Sigma-Aldrich (St. Louis, MO, USA), and ^{125}I-labelled hormones were obtained from Perkin-Elmer Life Sciences (Boston, MA, USA). Secondary antibodies were kindly provided by Dr. John McMurtry, USDA, Beltsville, MD, USA. The RIAs for T_4 and T_3 were validated for use with tree swallows samples by demonstrating parallelism between a series of diluted and spiked swallow plasma samples in relation to the standard curve. Duplicate tubes of two levels of Lymphocheck Immunoassay control serum were included with each assay to evaluate the consistency of assay performance. A precision test indicated that +/- 2SE was 3.1% of the mean for T4 and 2.6% of the mean for T3 (n=6 replicate samples). Recovery of hormone spikes added to quail plasma was 102% of T4 and 103% for T3. Interassay variation was less than 7% for both hormones. Assay sensitivity was 1.25 ng/ml for T4 and 0.125 ng/ml for T3.

4.3.4 Hormone Content in Thyroid Glands

Thyroidal hormone content was measured as described by McNabb and Cheng (1985), with minor modifications. Briefly, thyroid tissue was digested for 24h at 37 °C in 350 μL of digestion medium containing 25 mg of Pronase (Sigma-Aldrich Chemical, St-Louis, MO, USA). After digestion, 1.0 ml of absolute ethanol was added and tubes were vortexed to facilitate mixing. Hormones were extracted for 24h at -20°C and then centrifuged at 13,500 g for 5 minutes. An aliquot of the supernatant (containing the hormones) was removed and stored at -20°C until analysis. Dilutions of the supernatants were prepared in 75% ethanol and analyzed for T_4 and T_3 following the RIA assay protocol described above for plasma but using standards prepared in 75% ethanol. The optimal digestion time considering thyroidal protein digestion as well as preservation of released hormones was ~24 hours. At this time, 75% of radiolabel in thyroidal protein (prelabelled glands) had been released by the digestion. When labeled hormones were added to the homogenates, the extraction in ethanol recovered 100% of the labeled T4 and 96 % of the labelled T3. Precision of the RIAs with ethanol extracts was comparable to the plasma assays.

4.4.5 Statistical Analysis

Multilevel models are designed to analyze hierarchical data (clustered data). For instance, individuals clustered within households, clustered within different geographical areas can be surveyed using statistical equations that properly include all the appropriate dependencies (Hox, 2002, Dohoo et al., 2003). This approach has recently been used in ecological research with nestling birds (Simon et al., 2004). In this study, we used linear mixed models (one technique of multilevel analysis) to detect associations between SITE (DP, PC, NW, CT) and variables related to thyroid function, including a random intercept for “nest” in each model to account for the lack of independence among chicks from the same brood (PROC MIXED, SAS for Windows version 8.02, SAS Institute Inc., Cary, NC.). Level of significance was considered to be $P \leq 0.05$. Because ambient temperature and photoperiod can affect thyroid hormones (reviewed by McNabb, 2000) and those variables change during the course of the summer, hatch date was considered a potential confounding factor. Similarly, because larger nestlings may have larger thyroid glands and consequently more hormone content, body mass of nestlings was also considered a confounder. These were included as covariates in the models when $P \leq 0.05$ (or when inclusion of the factor in the model changed the P value by more than 10%).

4.4 RESULTS

4.4.1 Thyroid Hormones in Plasma

Concentrations of triiodothyronine (T_3) and thyroxine (T_4) in plasma are summarized in Table 4.1. Plasma T_3 (*P*=0.03), but not T_4 (*P*=0.1), increased with nestling body mass. Plasma hormones were not associated with hatch date (T_3 *P*=0.3; T_4 *P*=1.0). There were no significant differences among sites in concentrations of T_4 (*P*=0.09), the most abundant hormone in plasma. However, the most physiologically active hormone, T_3, was significantly higher in nestlings from CT ($P < 0.0001$) compared to those from PC or DP, and compared to those from NW (*P*=0.05). T_3 was also significantly higher in NW (*P*= 0.05) compared to control nestlings or to those from DP. The ratio T_3 / T_4 in plasma varied between 0.18 and 0.19 for all three sites except CT, on which it reached 0.31.

Table 4.1. Thyroid hormones (mean ± SD) in plasma of tree swallow nestlings on the four study sites in 2004

Study Site	**T_3 in plasma (ng/mL)**	**T_4 in plasma (ng/mL)**
Poplar Creek[1] (PC)	1.60 ± 0.37 A n=15	8.34 ± 2.79 A n=16
Demo Pond (DP)	1.37 ± 0.40 A n=16	7.53 ± 3.40 A n=16
Natural Wetlands (NW)	2.08 ± 0.59 B n= 12	10.71 ± 3.15 A n=12
Consolidated Tailings (CT)	2.57 ± 0.84 C n= 19	8.40 ± 2.06 A n= 19

T_3 = triiodothyronine, T_4 = thyroxine
[1] Reference site
Different letters indicate a significant difference ($P \leq 0.05$)

4.4.2 Thyroid Hormones in Thyroid Glands

Thyroid mass and hormone content within the glands are shown in Table 4.2. Thyroid mass was not associated with hatch date (*P*= 1.0), but heavier nestlings tended to have larger thyroid glands (*P*=0.03). Total thyroidal hormone content (ng per pair of thyroid glands) was not associated with nestling body mass (T_4 *P*=1.0; T_3 *P*= 0.9). As they hatched later in the season, nestlings had greater total thyroidal T_3 (*P*= 0.003) but not T_4 (*P*=0.9). The mass (*P*=0.09) of thyroid glands and their total thyroidal T_3 content (*P*=0.07) were not different among sites. However, total thyroidal T_4 content, which represents >95% of the hormones in thyroids, was higher in nestlings from two of the OSPM-impacted wetlands. The thyroid glands of nestlings from NW contained 1.8 times more T_4 than those from nestlings reared on the reference site PC (*P*=0.04). Similarly, nestlings from DP had 2 times more thyroidal T_4 than those from PC (*P*=0.003). Mean total T_4 on CT wetlands tended to be greater than on PC (Table 4.2), but differences were not significant (*P*=0.08).

Table 4.2. Thyroid hormones (mean ± SD) in the thyroid glands of swallow nestlings

Study Site	**Thyroid Mass (mg)**	**T_3 Content/ Glands (ng)**	**T_4 Content/ Glands (ng)**
Poplar Creek[1] (PC)	4.78 ± 1.29 A n= 16	0.90 ± 0.54 A n=16	24.02 ± 11.84 A n=16
Demo Pond (DP)	4.92 ± 1.41 A n= 16	1.44 ± 0.57 A n=16	47.65 ± 24.52 B n=16
Natural Wetlands (NW)	5.48 ± 0.79 A n=12	1.31 ± 0.48 A n=12	42.26 ± 19.80 B n=12
Consolidated Tailings (CT)	4.44 ± 1.03 A n=20	1.42 ± 0.60 A n=20	37.38 ± 18.95 AB n=20

T_3 = triiodothyronine, T_4 = thyroxine. Glands=pair of thyroid glands
[1] Reference site
Different letters indicate a significant difference ($P \leq 0.05$)

The thyroidal (i.e., within the glands) hormone concentrations (ng of hormones/mg of glandular tissue, reflecting weight specific hormone content) were not associated with nestling body mass (T_4 P=0.3; T_3 P= 0.1). Concentrations of thyroidal T_3 (P=0.01), but not T_4 (P=0.1) increased with hatch date. Compared to nestlings from the reference site, the concentrations of both thyroidal hormones were significantly higher in birds from two of the OSPM-impacted wetlands (Fig. 4.1). Thyroidal T_4 concentrations in nestlings from DP (P=0.005) and CT (P=0.03), as well as thyroidal T_3 concentrations in those from DP (P=0.03) and CT (P=0.004), were greater than in nestlings from the reference site PC.

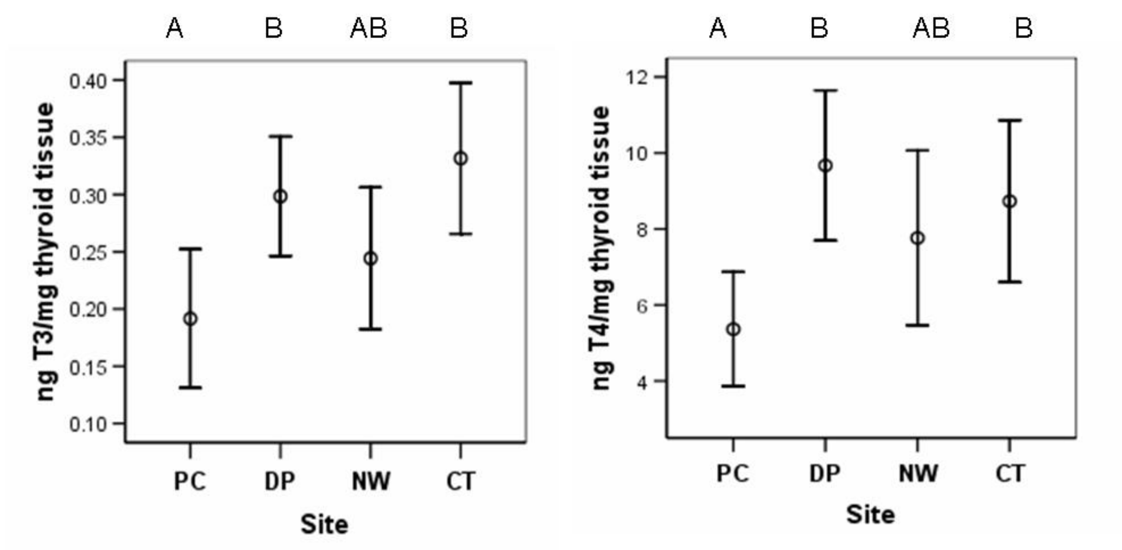

Fig.4.1. Concentration (ng hormone/mg thyroid tissue) of thyroidal triiodothyronine (T_3) and thyroxine (T_4) in the thyroid glands of tree swallow nestlings. Values are mean ± 95% confidence interval. PC= Poplar Creek (reference site), DP=Demo Pond., NW= Natural Wetlands, CT=Consolidated Tailings wetlands.

4.5 DISCUSSION

In this study, T_3 and T_4 were measured in tree swallow nestlings reared on sites containing waste materials from oil sands mining. Hormones were measured in plasma and in the thyroid glands, because the latter measurement is often the most sensitive indicator of altered thyroid function (reviewed by McNabb, 2005). Compared to nestlings from the reference site, those on Demo Pond (DP) had higher total thyroidal T_4 content, as well as higher thyroidal concentrations of T_3 and T_4. On Natural Wetlands (NW), nestlings exhibited greater T_3 in plasma and T_4 content within the thyroids, while on Consolidated Tailings wetlands (CT), they had higher concentrations of T_3 both in plasma and in the glands, as well as higher concentrations of T_4 in the glands. These results indicate that birds raised on OSPM-sites experienced an increase in stored T_4 within the glands, which could be either due to increased synthesis, decreased release, or a combination of the two. The lack of change in plasma T4 concentrations suggests that hormone release was not altered, but that thyroidal hormone synthesis was increased. The absence of thyroid gland hypertrophy on OSPM sites (as indicated by the lack of differences in thyroid weight among sites) is consistent with the lack of change in circulating T4. Thyroid gland hypertrophy normally results from activation of the hypothalamus-pituitary-thyroid axis. When circulating hormones decrease, the pituitary gland starts releasing TSH, which results in stimulation of all aspects of thyroid gland function, including thyroid growth (hypertrophy). Thus, our data suggest that the entire pituitary-thyroid axis was not targeted, but that some OSPM components were directly stimulating cellular thyroidal hormone synthesis without altering release from the gland. The increase in plasma T_3 on the two newest reclaimed sites is pointing toward an increase in extra-thyroidal conversion of T_4 into active T_3, i.e., increased deiodination. Whether the same chemical was increasing both deiodination in tissues and synthesis of T_4 within thyroid glands remains to be elucidated.

Since nestlings reared on reclaimed sites were exposed to a mixture of chemicals present in oil sands waste materials, which contain unrecovered bitumen, polycyclic aromatic hydrocarbons (PAHs) and dissolved organics such as naphthenic acids, it is not possible to determine which compounds were affecting thyroid function. The endocrine disrupting potential of naphthenic acids, a group of carboxylic acids that are toxic to aquatic biota (reviewed by Clemente and Fedorak, 2005) has never been investigated, thus their potential to disturb thyroid function is still unknown.

Ducks experimentally exposed to crude oil demonstrated an increase in thyroid hormone levels (Harvey et al., 1981, Peakall et al., 1981, Jenssen et al., 1990). Crude oils contain on average 0.2 to 7% polycyclic aromatic hydrocarbons (Neff, 1985), and there is substantial evidence from research with fish that PAHs may have been driving the increase in thyroid hormones in the above studies with waterfowl. When exposed to the water-soluble fraction of crude oil, which contains mostly PAHs, total body concentration of T_4 of larval and juvenile turbot increased (Stephens et al., 1997). Similarly, exposure to the PAH 3-methylcolanthrene increased plasma concentrations and thyroidal content of both T_3 and T_4 in Asian swamp eels *Monopterus albus* (Singh, 1989). Experimental injection of different individual PAHs into Amazon mollies (*Poecilia Formosa*) stimulated the proliferation of ectopic thyroid tissue in the spleen (Woodhead et al., 1982).

Polycyclic aromatic hydrocarbons are released during the weathering of petroleum (reviewed by Albers, 2003). Sediments of NW contained 2.54 (total parent PAHs) to 12.92 (total alkylated PAHs) times greater concentrations of PAHs than those from the Poplar Creek reference area in 1998 (Smits et al., 2000). Although preliminary work by Ganshorn (2002) was suggestive of low bioaccumulation potentials for PAHs in benthic and pelagic invertebrates, another study has documented sediment-to-invertebrate accumulation factors ranging from 1 to >100 in OSPM-impacted wetlands, and has reported significant levels of PAHs in adult insects emerging from those wetlands (Wayland and Smits, 2003). Therefore, it is possible that the increased levels of thyroid hormones observed in tree swallow nestlings documented in this study were associated with exposure to PAHs through dietary sources. The exact mechanisms by which PAHs or other chemicals on OSPM sites may affect thyroid function remain to be elucidated. It would be very valuable to expand biomonitoring of endocrine disruption to other species inhabiting OSPM-sites (including fish and amphibians) to see if patterns similar to what we documented in birds could be detected in aquatic biota. A histological evaluation of thyroid glands should be included to provide better understanding of the results, and the activity of specific thyroidal enzymes such as thyroperoxidases could be measured.

Our results suggest that other environmental factors, along with contaminants, are likely driving the increase in T_3 and T_4 because the data did not show a clear dose-response relationship. For instance, thyroid hormones were not always highest on the most-recently reclaimed site CT which contained the highest levels of NAs and presumably PAHs. It has been shown that food availability and dietary composition influence thyroid function in birds (reviewed by Sharp and Klandorf, 1985). Although previous research with tree swallows on our study area demonstrated that food boluses delivered to nestlings were qualitatively similar across sites (Smits et al., 2000), no study has investigated if nestlings on OSPM sites receive the same amount of food from their parents as those on areas unaffected by oil sands mining. Although logistically challenging, it would be valuable to measure food intake throughout the nestling rearing period, because total biomass of aquatic invertebrates varies between different OSPM sites (Whelly, 1999, Bendell-Young et al., 2000), which could affect the availability of emergent insects to swallows foraging on these sites.

Regardless of the cause, the altered thyroid function we documented in nestlings from OSPM-impacted sites is of concern because thyroid hormones are essential to the regulation of a large number of physiological processes. They influence basal metabolism and play a critical role in thermoregulation (Silva, 1995) as well as in normal development and growth (McNabb, 2000). Furthermore, in migratory birds, the onset and the termination of seasonal events such as breeding, molting and singing is triggered by changes in photoperiod and is partly controlled by thyroid hormones (reviewed by Dawson et al., 2001). Increasing day lengths in the spring initially stimulates the growth and maturation of the reproductive system (photostimulation), and at the end of the breeding period, an increase in thyroid hormone levels combined with long photoperiod eventually cause “photorefractoriness” to begin. Photorefractoriness involves the involution of the gonads and ensures the cessation of reproduction before environmental conditions deteriorate, allowing birds to prepare for migration (Jacobs and Wingfield, 2000). Thyroidectomy can prevent photorefractoriness from occurring, causing seasonal breeders to become continual breeders (Wilson and Reinert, 1993, Colborn, 2002), while treatment with thyroid hormones can mimic the effects of photorefractoriness, resulting in gonadal regression (Wilson and Reinert, 2000). Abnormal levels of circulating thryroid hormones can also precipitate molting or cause feather abnormalities (Lothrop 1996, Rae, 2000). Substantial changes in courtship and breeding behavior have been documented in doves with elevated thyroid hormones as a result from organochlorine exposure (McArthur et al., 1983). Those birds spent more time wing-flapping and less time nest building or brooding than control birds. As a result, they had lower reproductive success than non-exposed birds with normal thyroid hormone levels. In summary, considering that thyroid function controls such a large number of physiological processes, it is possible that nestlings fledged from OSPM-sites would have lower chances of surviving the post-fledging period and the fall migration, and would less likely to be recruited (i.e., survive to breeding) into the population than nestlings from sites unaffected by mining activities would. As mentioned previously, it would be very valuable to extend monitoring to the post-fledging period to verify this hypothesis. Unfortunately, this would be nearly impossible with tree swallows, because they do not usually return to the sites where they were reared for breeding, i.e., they have very high dispersal rates (Shutler and Clark, 2003).

4.6 CONCLUSIONS

In nestlings reared on reclaimed sites, plasma $T_{3,}$ total thyroidal T_4 (ng of hormones/glands) and thyroidal concentrations (ng of hormone/ mg of thyroid tissue) of both T_3 and T_4 were higher than in control nestlings. This excessive stimulation of thyroid function on sites impacted by Oil Sands Process-Materials could be associated with exposure to PAHs or other chemicals present in OSPM, but the confounding effects of other environmental factors such as food availability have yet to be investigated. Because thyroid hormones control such a large number of physiological processes, this modulation of thyroid function may result in lower chances for fledglings to be recruited (i.e., survive to breeding) into the population.

CHAPTER 5

Experimental Exposure of Nestling Tree Swallow to Naphthenic Acids

5.1 ABSTRACT

Naphthenic acids (NAs) are a group of carboxylic acids that are of particular concern to the steadily growing oil sands mining industry because they become highly concentrated in the water used for oil sands extraction and are toxic to aquatic biota and mammals. The toxicity of NAs alone to avian species has never been investigated. To address this void, tree swallow (*Tachycineta bicolor*) nestlings were dosed with 0.1 ml/day of NAs (15g/L) orally from day 7 to day 13 of age while being reared normally by their free-ranging parents. This represented a ten time "worst exposure" scenario for nestling tree swallows reared on oil sands reclaimed sites. Nestling growth, hematocrit, blood biochemistry, organ weights, and EROD activity were unaffected by naphthenic acids. The only change detected on histopathological evaluation of major organs was an increase in extramedullary erythropoiesis in the liver. These findings suggest that for nestlings reared on oil sands reclaimed sites, exposure to other chemicals such as polycyclic aromatic hydrocarbons is a greater concern than exposure to NAs. However, this study did not investigate the chronic or reproductive toxicity of naphthenic acids, and a previous study found that chronic exposure to NAs severely compromised reproduction in mammals. More research still needs to be conducted as a part of an assessment of the sustainability of wet landscape reclamation because birds will be breeding on reclaimed sites and will be exposed to NAs for several weeks.

5.2 INTRODUCTION

Naphthenic acids are a complex group of saturated mono-, poly- and acyclic carboxylic acids with surfactant properties that are naturally present in petroleum (Fig. 5.1). Naphthenic acids are of particular concern to the steadily growing oil sands mining industry in northern Alberta, Canada, which currently supplies about one-third of Canada's oil production (Alberta Department of Energy, 2005). The separation of crude oil from sand is accomplished by the Clark Hot Water Extraction process. This method involves the addition of sodium hydroxide (caustic soda) and large volumes of hot water (79-93 º C) to crushed oil sands to form a slurry (FTFC, 1995). The slurry becomes alkaline upon the addition of sodium hydroxide, and this promotes the liberation of naphthenic acids from bitumen (pKa ≈5). Since the same water is recycled several times during the extraction process, naphthenic acids become highly concentrated in the process-affected water. Once the bitumen has been recovered from the slurry, residual water, sand and clays, along with organic and inorganic contaminants, are diverted to large settling basins called tailings ponds (Mikula et al., 1996).

The Oil Sands Process-Affected Water (OSPW) has been proven toxic to a number of aquatic organisms (Mackay and Verbeek, 1993, Bendell-Young et al., 2000, van den Heuvel et al., 2000, Pollet and Bendell-Young, 2000). Naphthenic acids are responsible for most of the toxicity observed in these studies. In tailings ponds, concentrations of NAs can vary from 40 to 120 mg/L, but they typically range from 80-100 mg/L (Holowenko et al., 2002). Upon the completion of mining operations, OSPW will be incorporated into the environment. Since there is currently no discharge of the mining effluents from the sites, as much as 1 billion m^3 of oil sands tailings will require detoxification and reclamation (FTFC, 1995).

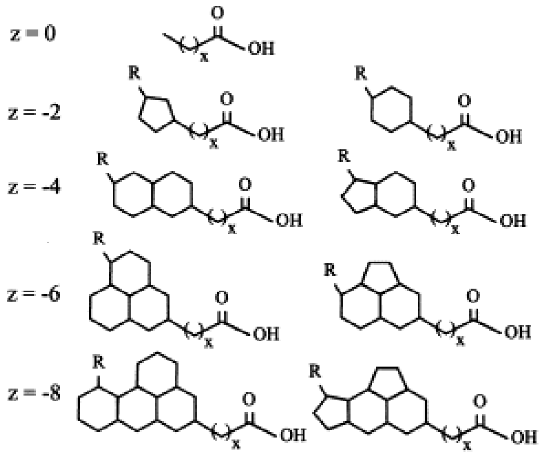

Fig. 5.1. Examples of naphthenic acid structures, where R is an alkyl group and *x* indicate the number of CH_2 groups in the carboxyl side-chain.

Information on NA toxicity to terrestrial vertebrates is very limited and restricted to a few acute exposure studies (LD_{50}) with laboratory rodents (Pennisi and Lynch, 1977, Lewis, 2000). Only one study investigated the effects of repeated exposure to NAs in mammals. Rogers (2003) found that a dosage just 10 times greater than the highest expected environmental exposure on reclaimed wetlands was sufficient to alter hepatic function and reproduction in rats. Dosing with 60 mg/kg/day NAs over a 90 day period resulted in excessive glycogen accumulation in the liver, elevated plasma amylase and depressed plasma cholesterol. The same dosage administered to females during the breeding period (7 weeks) impaired embryonic implantation, drastically reducing reproductive success: only 7% of females (1 of 14) produced a litter, compared to 100% and 93% in the low dose and control groups, respectively. The toxicity of NAs to avian species has never been investigated and needs to be characterized because birds colonizing reclaimed areas are exposed to those chemicals. In tree swallows (*Tachycineta bicolor*), exposure may occur from both the drinking water and the diet, because they feed mostly on insects of aquatic origin (McCarty and Secord, 1999, Wayland et al., 1998). Health assessments of tree swallows on oil sands reclaimed wetlands has been conducted previously (Smits et al., 2000), but the birds were exposed to a mixture of chemicals containing not only NAs but also other inorganic and organic compounds such as metals and polycyclic aromatic hydrocarbons. In this field-based exposure study, cohorts of wild tree swallow nestlings received doses of NAs orally, but were otherwise naturally reared by their free-ranging parents. This approach combines the advantages of controlled exposure conditions with the relevance of using a native species in its natural habitat.

5.3 METHODS

5.3.1 STUDY DESIGN

5.3.1.1 Study Site

Poplar Creek (PC) is a 2.5 km x 0.5 km reservoir located approximately 10 km south of active mining sites (57° 00' N, 111° 30'E). It was created in 1975 when a river flowing through the mining areas had to be diverted prior to beginning operations. It does not receive any mining effluents. This site was chosen because of its proximity to the mining leases, ensuring that habitat and weather would resemble what is encountered on the oil sands areas.

5.3.1.2 Study Animals

A colony of tree swallows has been monitored on PC since 1997. There are 26 nest boxes on this site, which are spaced from each other by 10-20m. Nest boxes are made of wood and measure 16 cm (width) x 13 cm (depth) x 25.3 cm (height). One of the walls is equipped with hinges so the box can easily be opened, providing access to the nest. The nest boxes are located on the grassy shore of the reservoir and are mounted on 1.5 – 2m metal posts. The entrance holes face south. Tree swallow activity was monitored from May 19^{th} to July 15^{th} 2004. Each nestling within a brood was uniquely identified with different nail color combinations. Nestlings that were destined to terminal sampling, (i.e., those that were dosed with NAs or saline, plus a subset of 16 unmanipulated controls) were collected at 14 days of age. They were anesthetized in a container with Halothane (MTC Pharmaceuticals, Cambridge, Ontario, Canada) and then euthanized by cervical dislocation.

5.3.1.3 Experimental Groups

Ten nests with ≥5 nestlings were randomly selected for the experimental exposure study. Nests selected for the experimental exposure were excluded from the study of "natural exposure to OSPM", i.e., data from those nests were not analysed in chapters 2 and 3. Within each brood, two nestlings were randomly assigned to receive naphthenic acids (n=20) and two nestlings were randomly assigned to receive the same volume of a saline solution (n=20). Birds assigned to the "NAs" group received 0.1 ml of a 15 g/L aqueous solution of naphthenic acids (Pfaltz and Bauer Chemicals, Waterbury, CT, USA) provided by Dr Mike MacKinnon from Syncrude Ltd. while those assigned to the "saline" group received 0.1 ml of 0.9% NaCl (EMD Chemicals Inc., Gibbstown, N.J.). Doses were delivered into the crop (by gavage) with syringes equipped with a smooth, blunted plastic tip. Nestlings were dosed once a day from day 7 to day 13 post-hatch (hatch day = day 1). To detect whether the stress associated with the gavage procedure rather than the treatment itself was affecting the birds, nestlings (from experimental and non-experimental nests) that were not receiving any treatment were also included in the study as "unmanipulated controls" (n=16).

5.3.1.4 Calculation of the Dosage for NA Exposure

Feeding rates of nestlings may vary with location, so we used data from research conducted in Alberta for dosage calculation. Dunn et al. (1992) found the food boluses delivered to tree swallow nestlings to weigh on average 45mg. Adults bring an average of 18.2 boluses /hr to the nest and forage for about 15 hrs a day (McCarty, 2002). Since the average brood size of tree swallows is 5.5, each nestling would receive approximately 2.2 g of insect /day. It is not possible to quantify NAs in biological tissues with the current analytical techniques, and there are no data available on uptake and elimination rates of NAs within invertebrates. Thus insects were estimated to contain the same concentration of NAs as water of a newly reclaimed wetland, such as our study site CT wetlands in which they average 68 mg/L (Golder Associates, 2004). Therefore, nestlings on reclaimed sites would consume approximately 0.15 mg naphthenic acids/day through their diet. Because both McCarty (2002) and Dunn et al. (1992) found that tree swallow parents do not seem able to adjust feeding rates of their young according to factors such as nestling age, size and brood size (e.g., nestlings do not necessarily receive more food as they grow and parents do not necessarily bring more food to larger or older broods, etc.) daily exposure was considered constant throughout the study. Thus, to simulate a 10 times “worst-case” exposure scenario on reclaimed wetlands, nestlings received 1.5 mg NAs/day.

5.3.2 ENDPOINTS

5.3.2.1 Nestling Growth

Nestling mass was measured to the nearest 0.05g using a Pesola spring balance and wing length (carpus to end of longest primary feather, allowing the wing to rest naturally on the ruler) was measured to the nearest 0.1cm. Growth was measured on day 6, 9, 12 and 14 (hatch day = day 1).

5.3.2.2 Hematology and Blood Biochemistry

Blood was collected by cardiac puncture from anesthetized nestlings just before euthanasia. One drop of blood was collected in heparinized capillary tubes to measure hematocrit (packed cell volume, PCV). After centrifugation, the plasma obtained from those capillary tubes was used to measure total proteins (Reichert TS refractometer). Remaining blood was centrifuged separately and plasma was frozen in individual vials at –20°C. Blood biochemistry analysis was carried out on plasma at the clinical pathology laboratory of the Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon (SK), Canada.

5.3.2.3 Ethoxyresorufin-O-Deethylase (EROD) Activity

The activity of this enzyme was measured to assess the intensity of hepatic detoxification efforts. Please refer to section 2.3.4 for the methods used to measure EROD activity.

5.3.2.4 Organ Weight and Histological Examination of Tissues

Major organs (kidney, spleen, heart, ileum, thymus, bursa of Fabricius) were removed within 15 minutes of death. The weight of liver, heart, spleen and bursa was measured to the nearest 0.001 g. In this thesis the somatic index (S.I.) is also reported for each organ, calculated as organ weight / nestling body mass. After being weighed, all organs (except the left lobe of the liver which was used to measure EROD) were fixed in 10% buffered formalin. Sections of the fixed organs were stained with hematoxylin and eosin at the histology laboratory of the Western College of Veterinary Medicine, Saskatoon (SK), Canada. The observer was blinded to the origin of tissues when conducting histological examinations. All tissues were examined for parasites, inflammation, necrosis or other pathological changes.

5.3.2.5 Detailed Evaluation of Lymphoid Organs

Spleens were cut longitudinally and the entire section was examined (10X magnification) to count the number of active germinal centers. The density of apoptotic lymphocytes (number of apoptotic cells per field) in the bursa of Fabricius was initially evaluated in a subsample of nestlings, but this gave nearly identical results for every bird because all bursas were well populated by lymphocytes, with no evidence of involution or excessive lymphocytolysis. Thus this endpoint was removed from the protocol and replaced with measurements of cross-sectional area (mm^2) covered by lymphoid tissue (lymphocytes). This was carried out on histological slides (2X magnification) using image analysis software (Northern Eclipse, version 6.0, 2002, Mississauga, ON, Canada). It provided a more meaningful evaluation of bursal development than organ weight or somatic index, because image analysis software allowed precise measurements which excluded connective and adipose tissue. It also allowed the exclusion of the empty space inside the bursa from the measurements (the bursa is a tubular organ).

5.3.2.6 Detailed Evaluation of the Liver

Because previous research showed the liver to be a target for NAs toxicity (Rogers 2003), this organ was examined using several criteria (Table 5.1) that were chosen to qualify liver morphology (hepatocellular vacuolation), as well as to quantify normal hematopoiesis. In young mammals, the bone marrow supports the development and maturation of red and white blood cells, but in birds, this role is also assumed by the liver (Wong and Cavey, 1993). Developing heterophils and eosinophils (extramedullary granulopoiesis) usually aggregate around periportal spaces, forming clusters that can be counted at 10X magnification (Fig. 5.4). Immature red blood cells are intensely basophilic and can be detected in liver sinusoids at 2X magnification (Fig.5.4). When hepatocellular vacuolation was detected in the liver, further testing was carried out to determine if it was caused by glycogen (Periodic Acid-Shiff stain, with and without prior diastase digestion) or lipid accumulation (Oil-Red-O stain).

Table 5.1. Histopathological evaluation of the liver in nestlings exposed to naphthenic acids

Parameter	Aspect Evaluated	Score System	Description of Ranking Criteria
Hepatocellular vacuolation [1]	Type	F= feathery	Small vacuoles are irregular (ground-glass or window frost appearance), in severely affected cells the nucleus is suspended between narrow strands of bridging cytoplasm.
		R= round	Vacuoles are round and nucleus is pushed aside in most affected cells
	Intensity	0 = none	Vacuolation is minimal or absent
		1 = mild	<50 % of hepatocytes have vacuoles and vacuoles are small (occupying <50% cytoplasm)
		2 = moderate	50-75% hepatocytes have vacuoles and vacuoles occupy about half of the cytoplasm
		3 = marked	Nearly 100% hepatocytes are vacuolated, vacuoles fill most of the cytoplasm and distend hepatocytes
Granulopoiesis [2]	Intensity	Cluster density	The whole slide was scanned to count the number of clusters of immature heterophils. Aggregates comprising about 50 cells were considered a cluster.
Erythropoiesis [3]	Intensity	1 = mild	Scant immature erythrocytes in liver sinusoids
		2 = moderate	Obvious immature erythrocytes in liver sinusoids
		3 = marked	Marked presence of immature erythrocytes, with distension of sinusoids

[1] Examination of 10 random fields at 20X and 40X magnifications
[2] Number of clusters was recorded for each microscopic field examined at 10X magnification, covering the whole slide. Mean density of granulocyte clusters was calculated as total # of clusters in entire liver section / total number of fields examined
[3] Whole slide examined at 2X magnification

5.3.3 Statistical Analysis

Data from nestlings of a same brood exhibit some degree of correlation. Unlike most statistical tests, which rely on the assumption that all observations must be independent, multilevel models are designed to analyze hierarchical data (clustered data). For instance, individuals clustered within households, clustered within different geographical areas can be surveyed, using equations that properly include all the appropriate dependencies (Hox, 2002, Dohoo et al., 2003). This approach has recently been used in ecological research with nestling birds (Simon et al., 2004). In this study, we used linear mixed models (one technique of multilevel analysis) to detect associations between TREATMENT (NAs, saline, unmanipulated) and continuous outcomes (e.g., EROD, organ weight, hematology and biochemistry data), including a random intercept for "nest" in each model to account for the lack of independence among nestlings from the same brood (PROC MIXED, SAS for Windows version 8.02, SAS Institute Inc., Cary, NC.). For the analysis of growth (body mass, wing length), models accounting for two hierarchical levels were used (multiple observations within individuals, individuals clustered within nests). The associations between treatment and discrete outcomes were similarly analyzed using generalized estimating equations (PROC GENMOD, SAS for Windows version 8.02). Discrete data (lymphoid nodules in the spleen) were analyzed using Poisson distribution models with a with log link function. Hepatic vacuolation scores were converted into binomial data (scores 0 and $1 \rightarrow 1$; scores 2 and $3 \rightarrow 2$) because the sample size was too small for multinomial data analysis with SAS and these binomial data were then analyzed using logistic regression models with a logit link function. Hepatic erythropoisesis scores could not be converted into binomial data (because rated 1, 2 or 3) so the original scores were compared using the computer software MLwiN 10.0 (Centre for Multilevel Modelling, London, UK). The threshold for statistical significance for all statistical tests was $P \leq 0.05$.

5.4 RESULTS

Eight of the 40 dosed nestlings (NAs and saline) were killed by a black bear who ventured onto the study site and destroyed nest boxes. One nestling in the saline group died; no lesions or abnormalities could be detected upon gross and histological post-mortem examination.

5.4.1 Nestling Growth

As in many bird species, the weight gain of nestling tree swallows was curvilinear (Fig.5.2), while wing growth was linear (Fig.5.3). As illustrated on figures 5.2 and 5.3, there was no consistent trend between exposure groups, in fact, all nestlings had almost identical growth rates. Adjusted to a common age (age: $P<0.0001$), neither nestling weight ($P=0.9$) or wing length ($P=0.3$) varied among treatments.

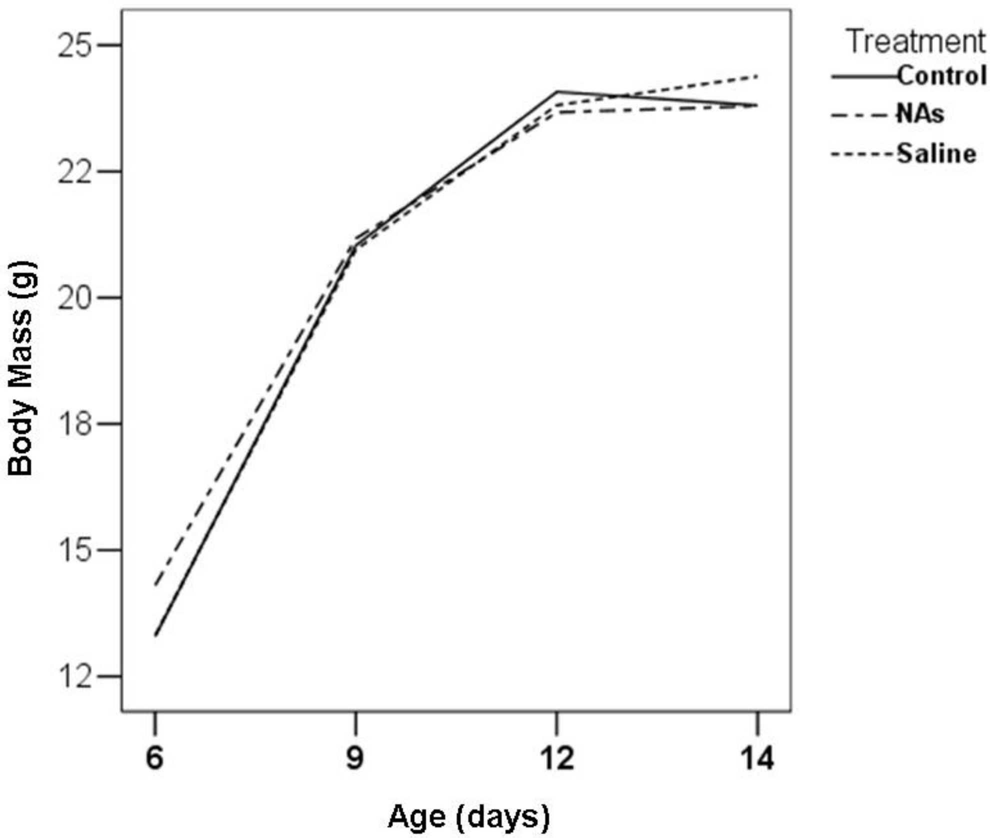

Fig. 5.2 Weight gain of nestling tree swallows from different treatment groups. Birds were orally dosed with NAs or saline, or were left unmanipulated (i.e. control)

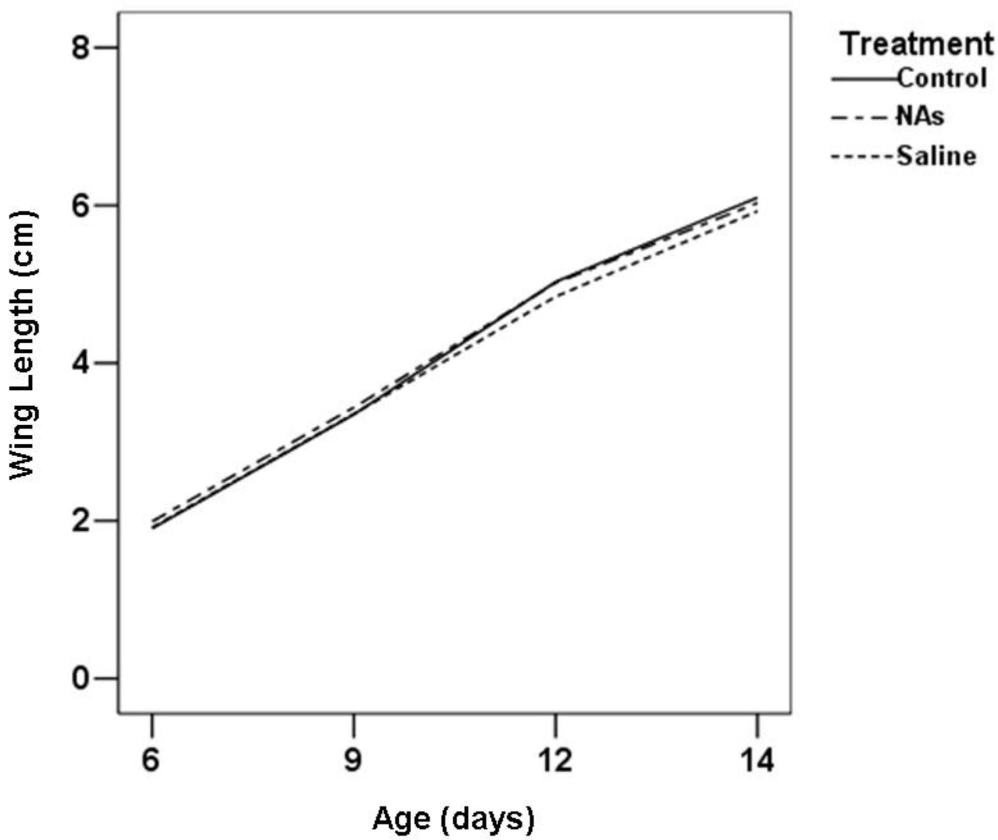

Fig.5.3 Wing growth of nestling tree swallows from different exposure groups. Birds were orally dosed with NAs or saline, or were left unmanipulated (i.e. control)

5.4.2 Hematology and Blood Biochemistry

There were no significant differences (*P*=0.4) in hematocrit (packed cell volume, PCV) among treatments (Table 5.2). However, compared to control nestlings, those exposed to naphthenic acids (*P*= 0.02) and saline (*P*= 0.04) showed a significant increase in total blood proteins of 0.54 g/L and 0.14 g/L, respectively. When the data were analyzed separately for each treatment, total proteins were positively associated with hematocrit in birds exposed to saline (*P*= 0.009) and NAs (*P*= 0.002), but not in control birds (*P*=0.8).

Table 5.2. Hematology values (mean ± SD) of tree swallow nestlings receiving one of three treatment groups in an experimental exposure study to naphthenic acids in 2004

	Control [1]	**NAs**	**Saline**
Hematocrit (%)	44.13 ± 4.46	47.43 ± 8.95	44.07 ± 8.29
	n=16	n=14	n=15
	A	A	A
Total proteins (g/L)	4.21 ± 0.36	4.75 ± 0.69	4.35 ± 0.57
	n=16	n=15	n=15
	A	B	C

[1]Unmanipulated controls
Different letters indicate a statistically significant difference ($P \leq 0.05$)

Although blood biochemistry results presented in Table 5.3 suggests a marginal increase in nearly all blood biochemistry values (except creatine kinase) for nestlings exposed to NAs compared to control nestlings, differences between treatments were not significant (cholesterol: *P*= 0.3; glutamate dehydrogenase: *P*= 0.7; creatine kinase: *P*= 0.6; aspartate aminotransferase: *P*=0.2; bile acids: *P*= 0.4; glucose: *P*= 1.0; uric acids: *P*= 0.7). There was considerable variation in blood biochemistry values among birds in a same treatment group. This was particularly evident for AST, as reflected by a standard deviation much greater than the mean (Table 5.3). This was not driven by one outlier, but rather by the wide range of values measured for this enzyme, which should be expected for a wild animal species.

Table 5.3. Blood biochemistry (mean ± SD) of tree swallow nestlings receiving one of three[1] treatment groups in an experimental exposure study to naphthenic acids in 2004

	Control[2]	**NAs**
Cholesterol (Mmol/L)	4.85 ± 0.56 n=16	5.21 ± 0.95 n= 9
GLDH (U/L)	8.94 ± 5.97 n=16	10.00 ± 8.93 n=9
CK (U/L)	589.69 ± 225.575 n=16	535.56 ± 122.99 n=9
AST (U/L)	346.29 ± 173.15 n= 16	963. 54 ± 1715.60 n=13
Bile Acids (Umol/L)	3.00 ± 1.66 n=16	3.62 ± 2.40 n=13
Glucose (Mmol/L)	17.19 ± 1.76 n=16	17.22 ± 2.32 n=13
Uric Acids (Umol/L)	690. 19 ± 201.25 n=16	709.11 ± 236.23 n=9

[1]Blood biochemistry values for the saline group were not measured
[2]Unmanipulated controls
GLDH= glutamate dehydrogenase, CK= creatine kinase, AST= aspartate aminotransferase

5.4.3 EROD and Organ Weights

Liver detoxification efforts (Table 5.4), as reflected by ethoxyresorufin-*o*-deethylase activity (EROD), did not significantly change among treatments (P=0.1). Mean weight of major organs (Table 5.4) did not vary between exposure groups (liver:

P=0.9; spleen: P=1.0; heart: P=0.8; bursa: P=0.4). Somatic indexes (S.I. =organ weight/ nestling body mass) were also examined, but there were no significant differences among treatments (liver S.I.: P=0.4; spleen S.I.: P= 0.9; heart S.I. P=0.3; bursa S.I. P=0.6).

Table 5.4 Organ weights, somatic indexes and EROD[1] of tree swallow nestlings in an experimental exposure study to naphthenic acids in 2004

		Unmanipulated Controls	**Naphthenic Acid Exposure**	**Saline Exposure**
Liver	weight	1.26 ± 0.22 n= 16	1.31 ± 0.30 n= 16	1.28 ± 0.26 n= 14
	S.I.	0.052 ± 0.010	0.055 ± 0.010	0.052 ± 0.008
Heart	weight	0.317 ± 0.044 n= 16	0.317 ± 0.028 n= 16	0.309 ± 0.042 n= 15
	S.I.	0.013 ± 0.001	0.013 ± 0.002	0.013 ± 0.001
Spleen	weight	0.044 ± 0.029 n= 16	0.044 ± 0.021 n= 16	0.044 ± 0.021 n= 16
	S.I.	0.0018 ± 0.0013	0.0018 ± 0.0008	0.0017 ± 0.0008
Bursa	weight	0.075 ± 0.020 n= 16	0.065 ± 0.019 n= 15	0.068 ± 0.018 n= 15
	S.I.	0.0030 ± 0.0007	0.0028 ± 0.0007	0.0028 ± 0.0007
Hepatic EROD Activity		38.03 ± 23.22 n=16	20.84 ± 16.43 n=16	30.66 ± 23.49 n=15

Results = mean ± SD. All organ weight expressed in grams (g)
S.I. = somatic index (organ weight/ nestling body mass)
[1]Ethoxyresorufin-o-deethylase (EROD) activity expressed as pmol/min/mg microsomal protein

5.4.4 Histological Examination of Major Organs

One nestling exposed to NAs had a small abscess in the bursa of Fabricius (encapsulated cluster of degenerated heterophils obliterating one follicle). This was likely an incidental finding that was not related to treatment. Apart from this, there was no evidence of inflammation, necrosis or parasites in the liver, kidney, spleen, heart, ileum, thymus or bursa of Fabricius in any nestling.

5.4.5 Detailed Evaluation of Lymphoid Organs

Spleens of all nestlings appeared inactive, i.e. germinal centers were scant and very poorly defined. The number of germinal centers in a spleen increased with spleen weight (P=0.05). There were no differences among treatments in the number of germinal centers counted on longitudinal sections of the spleen (P= 0.2, adjusted to common spleen weight). In all nestlings, the bursa of Fabricius appeared healthy and follicles were densely populated with lymphocytes. There was no evidence of involution or excessive lymphocytolysis in any of the bursas. Measurements of cross-sectional area (mm^2) covered by lymphoid tissue increased proportionally with nestling weight (P=0.02). Because nestling weight may act as a confounder and because we wanted to specifically assess the effects of NA exposure on the bursa, we included body mass as a covariate in the model. Bursa area, adjusted to common body mass, did not significantly vary among treatments (P=0.9). Measuring the area covered by lymphoid tissue (Table 5.5) produced results slightly different from those obtained from weighing the bursa (Table 5.4). For instance, unmanipulated nestlings had the heaviest bursa, but the largest (in mm^2) bursa area was found in nestlings dosed with saline. Using computerized image analysis was more precise than using organ weight or somatic index to compare treatments, because it excluded connective and adipose tissues which are often difficult to remove during dissection. Furthermore, all measurements of bursal area were carried-out by the same examiner (me), whereas excision and weighing of this organ in the field was done by up to three assistants with different dissection skills.

Table 5.5. Evaluation of lymphoid organs (mean ± SD) of nestling tree swallows in an experimental exposure study to naphthenic acids in 2004

	Area Covered by Lymphocytes in Bursa of Fabricius[2]	**Number of Germinal Centers in Spleen[3]**
Control[1]	6.59 ± 1.48 n=12	7.67 ± 9.08 n=15
NAs	6.77 ± 1.91 n=16	4.80 ± 9.33 n= 15
Saline	6.92 ± 1.81 n=14	6.80 ± 9.98 n=15

[1]Unmanipulated controls
[2]Total area (mm^2) measured on cross-section using image analysis software
[3]Total number of germinal centers in a longitudinal section of the spleen, counted at 10X magnification

5.4.6 Detailed Evaluation of the Liver

Livers exhibited various cellular changes (Table 5.6). Most birds (91.5%) had at least some degree of feathery hepatocellular vacuolation (score 1, 2 or 3). Mild or moderate vacuolation was most common (40.4% scored 1; 31.9% scored 2), but marked vacuolation (scored 3) was detected in 19% of the birds. In livers with marked feathery vacuolation, hepatocytes were distended by multiple vacuoles that occupied considerable portions of the cell and the nucleus often appeared suspended between narrow irregular strands of bridging cytoplasm (Fig.5.5). Glycogen accumulation in hepatocytes with feathery vacuolation was confirmed with PAS staining (Fig. 5.6). Round vacuolation was less common (27.7% of the birds scored 1, 2 or 3) and only occurred in livers that also exhibited feathery vacuolation. These round vacuoles, typical of lipid accumulation (confirmed with Oil-Red-O), were easily distinguishable from those containing glycogen. There were no associations between treatment and degree (score) of feathery (P=0.4) or round (P=0.6) vacuolation in the liver. Livers showed substantial extramedullary erythropoiesis and granulopoiesis (Fig. 5.4 and Table 5.6). There were no differences among treatments in the numbers of granulocyte clusters per microscopic field (P=0.9). However, erythropoiesis scores were significantly higher in nestlings dosed with NAs than in control birds (P=0.001).

Table 5.6. Histopathological evaluation of the liver of nestling tree swallows in an experimental exposure study to naphthenic acids in 2004

	Vacuolation [2]		**Erythropoiesis** [3]	**Granulopoiesis** [4]
	Feathery	Round		
Control[1]	1.56 ± 0.81 n=16	0.69 ± 1.078 n=16	1.50 ± 0.63 A n=16	3.02 ± 2.75 n=16
NAs	1.56 ± 1.03 n= 16	0.44 ± 0.81 n=16	2.00 ± 0.63 B n=16	3.17 ± 3.57 n=16
Saline	1.73 ± 0.88 n=15	0.60 ± 1.06 n=15	1.73 ± 0.70 A n=15	2.60 ± 2.05 n=15

[1] Unmanipulated controls
[2] Mean vacuolation score (0=none, 1=mild, 2=moderate, 3=marked)
[3] Mean erythropoiesis score (1=mild, 2=moderate, 3=marked)
[4] Mean number of granulocyte clusters/ microscopic field
Different letters (erythropoiesis column) indicate a statistically significant difference ($P \leq 0.05$)

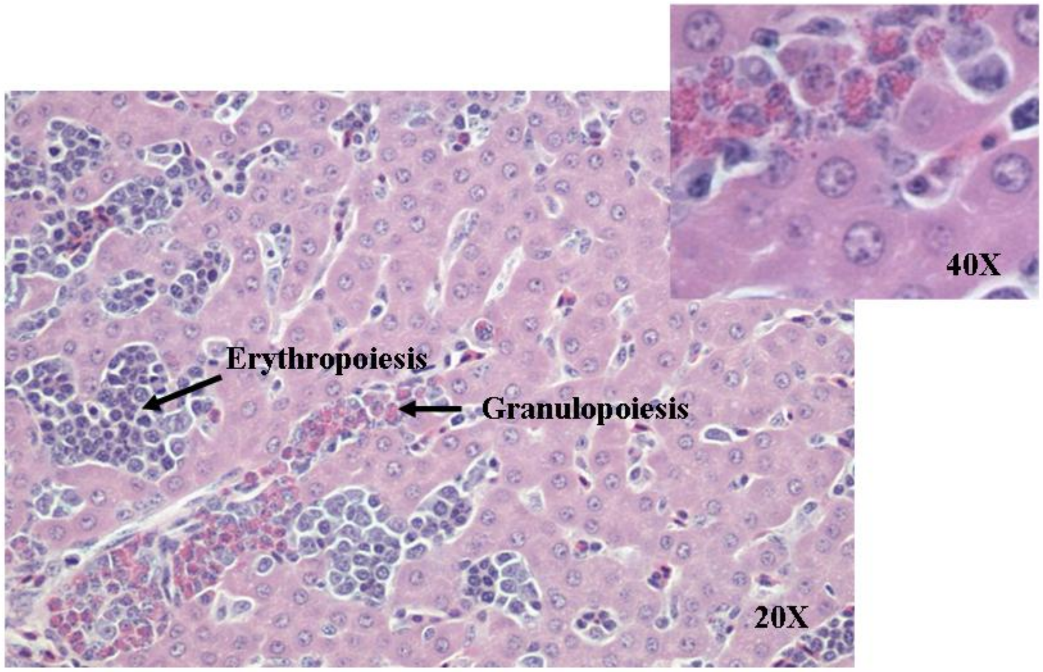

Fig. 5.4 Normal liver of nestling tree swallow (score 0)

H&E, 20X & 40X magnification

The cytoplasm of hepatocytes is devoid of vacuoles. Immature heterophils (granulopoiesis) form clusters around a periportal space and immature erythrocytes (erythropoiesis) fill the sinusoids. The 40X magnification shows cellular structure in more details. The red granules of granulocytes are very apparent at this magnification.

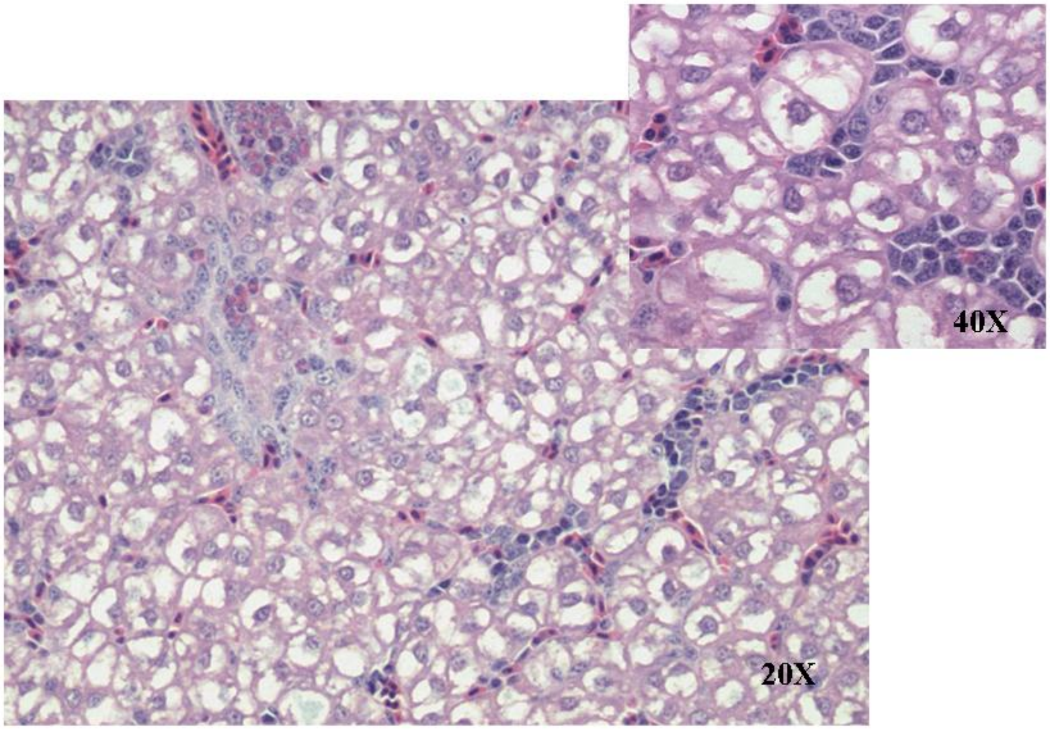

Fig. 5.5 Vacuolated liver of nestling tree swallow (score 3)

H&E, 20X & 40Xmagnification
Large feathery vacuoles fill the cytoplasm of hepatocytes. The nuclei seem suspended between strands of bridging cytoplasm.

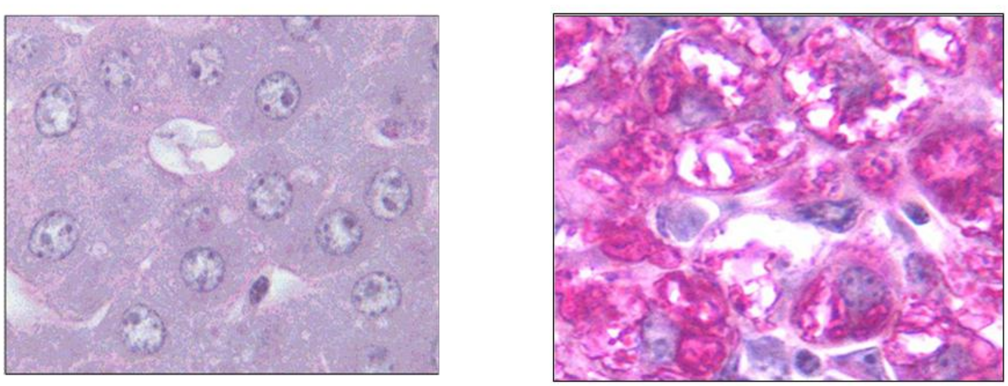

Fig. 5.6 Glycogen accumulation in hepatocytes of nestling tree swallow

Staining with Periodic Acid Shaff (PAS) gives glycogen a magenta color. The picture on the left is PAS negative, the picture on the right is PAS positive. 40X magnification.

5.5 DISCUSSION

5.5.1 Major Findings

Our results contrast with those from Rogers (2003), who conducted NA exposure studies in rats and documented significant effects on liver function and reproduction. There was generally little indication that nestlings suffered pathological effects from the exposure to naphthenic acids. There were no associations between treatment and endpoints such as growth, hematology, blood biochemistry, organ weights and EROD activity. Histopathological evaluation of major organs did not reveal changes related to exposure, except for an increase in erythropoiesis scores in the liver. It is very difficult to determine whether or not this was a toxic response and the implications of these findings for nestling health remain unclear. Very little is known about the mechanisms controlling hematopoiesis in birds. In the mammalian fetus, red blood cells first develop in close association with hepatocytes (extravasculary) and must gain entry to the sinusoids secondarily (Asano et al., 1980). Wong and Cavey (1993) hypothesized that a similar migration of erythroblasts through gaps in the sinusoidal walls would occur in birds. Maizelis et al. (1980) documented increased permeability of capillaries in rats following exposure to naphthenic acids. Perhaps exposure to NAs increases the size of gaps in sinusoidal walls, allowing larger numbers of erythroblasts to migrate and accumulate in sinusoids. Alternatively, perhaps naphthenic acids damaged mature circulating red blood cells and decreased their life span, and the increased hepatic erythropoiesis reflected higher demand associated with higher turnover. In order to better characterize the effects of NAs on avian erythropoiesis, the findings of this study should first be confirmed by precisely measuring erythroblast density in the liver using computerized image analysis, which would be more objective than a scoring system. If this produced significant results, research should be undertaken to understand the pathogenesis.

Total plasma proteins were significantly elevated in nestlings exposed to NAs and saline, but this was likely not related to toxicity. An increase in both PCV and total plasma proteins is highly suggestive of dehydration (Jain, 1986), which in this case was presumably due to the sodium content of both dosing solutions. Naphthenic acids were available as an aqueous solution of sodium salts, and the sodium concentration of the saline solution was adjusted to approximately reflect that of the NAs solution in order to investigate NAs toxicity without the confounding effect of salt intake. Rogers (2003) observed an increase in water consumption in rats chronically exposed to high doses of NAs, and attributed this behavior to an attempt to relieve dehydration.

The detailed histopathological examination of the liver conducted in this study was undertaken because previous research showed this organ to be the main target of toxicity. While hydropic or fatty degeneration are the most common changes observed in the liver following mild and moderate toxic insults (Jubb and Kennedy, 1970), diffuse swelling of hepatocytes with vacuoles containing mostly glycogen is occasionally seen after mild liver injury (Dwivedi and Burns, 1984, Nayak et al., 1996, Thophona et al., 2003). Rats chronically exposed to NAs exhibited abnormal hepatic vacuolation due to excessive accumulation of glycogen (Rogers et al., 2002a), and it was of interest to see if this would be seen in birds. Dokholyan and Magomedov (1983) also detected an increase in glycogen content of muscle tissue in fish chronically exposed to NAs, suggesting that this group of chemicals may disrupt enzymatic pathways involved in glycogenolysis or glycogenesis. In this study, various degrees of hepatocellular vacuolation were observed in liver tissue of nestling tree swallows, and the vacuolation was indeed mostly caused by glycogen accumulation. However, mean vacuolation scores were not different among exposure groups (in fact they were identical for unmanipulated control and NAs-exposed birds). This implies that variations in hepatocellular vacuolation observed in this study were associated with normal carbohydrate metabolism rather than NA toxicity. Hepatocyte morphology is highly variable depending on nutritional state: glycogen accumulates in the cytoplasm after consumption of a meal, and is depleted relatively rapidly after a period of fasting (Hammad et al., 1982).

5.5.2 Hypothesis Explaining the Lack of Toxic Response to NAs

This research indicates that a 10 times "worst exposure" scenario to NAs for 7 days is not toxic to nestling tree swallows. A number of factors may explain the absence of measurable toxic response in this study. Unlike aquatic organisms, which are relatively sensitive to NAs and suffer pathological effects when exposed to low concentrations in water (Patrick et al., 1968, Dokholyan and Magomedov, 1983, Davis, 1992), birds may be able to tolerate environmentally realistic doses of NAs. Aquatic organisms are highly susceptible to the toxic action of surfactants on skin and gills. The amphiphilic molecular structure (i.e., both hydrophilic and hydrophobic) of surfactants allows them to be most active at the oil-water, air-water and solid-water interfaces, where they modify surface tension and alter electron transfer. Surfactants can insert themselves into cellular membranes (because their structure resembles that of phospholipids, which are the main components of cellular membranes) and cause dysfunction; they also readily bind to polypeptide chains, altering the function of proteins and enzymes (reviewed by Cserháti et al., 2002). In aquatic organisms, surfactants increase mucus production in the epidermis and gills. They also damage the epithelium of the gills and alter mitochondrial structure, which results in hypoxia and death (Lewis, 1991, Cardellini and Ometto, 2001, Mann and Bidwell, 2001). It has been suggested that the surfactant properties of NAs (section 1.3.2) may be responsible for most of the toxicity in aquatic species (Quagraine et al., 2005, Schramm et al., 2000). The mechanism of NAs toxicity is likely different in terrestrial biota because the route of exposure is different: exposure would be through ingestion rather than from direct contact on skin and gills at the water-solid interface. Since NAs show a degree of similarity with natural fatty acids (Clemente and Fedorak 2005) and bile acids (Rogers, 2003), perhaps higher vertebrates ingesting NAs would be able to metabolise them. However, the results of previous studies which revealed toxic effects of ingested NAs in rodents challenge this hypothesis (Pennisi and Lynch, 1977, Rogers et al., 2002a). There is a possibility that laboratory rats which have been bred and reared in controlled environments for generations would be more sensitive to naphthenic acid toxicity than wild birds, which are constantly adapting to changing environmental conditions. Also, the naphthenic acids used by Rogers et al., (2002a and 2002b) had been extracted from oil sands effluents whereas ours were from a commercial source, so the chemical composition of dosing solutions was different between the two studies. However, the toxicity of both NA mixtures should have been comparable, because commercial NAs show fingerprints similar to those of NAs from fresh tailings in the main holding pond on Syncrude, Mildred Lake Settling Basin (Clemente et al., 2003). Rogers extracted his NAs from tailings collected in Mildred Lake settling basin (Rogers et al., 2002b).

The results from our study do not actually contradict those from mammalian studies. We found that nestlings tree swallows were able to tolerate daily exposures to 1.5 mg NAs without major detrimental effects on health. Since nestling weight between day 6 and day 14 post-hatch averaged 20g, exposure would have been approximately 0.075 g/kg throughout the study. Considering that the LD_{50} for laboratory rodents ranges from 3 to 3.55 g/kg (Pennisi and Lynch, 1977, Lewis, 2000) and that Rogers found no toxic response in rats acutely exposed to dosages below 0.3 g/kg, it was to be expected that a dosage of 0.075g/kg would only elicit minimal reactions in birds (if NAs were metabolized following similar pathways in both vertebrate classes). This dosage was chosen to reflect environmentally relevant concentrations of NAs. It is very possible that exposure to higher doses of NAs would have altered nestling's health; however, it would be difficult to extrapolate the findings from such a study to oil sands mining reclamation scenarios. It would also be physically impossible to investigate the toxicity of larger doses of NAs to nestling tree swallows. Naphthenic acids cannot be maintained in solution at high concentrations (they precipitate), and it was found in a pilot study that larger volumes of dosing solution induced osmotic diarrhea in nestlings.

The results from this study indicate that subacute (7 days) exposure to NAs does not harm nestling tree swallows. Thus, based on these findings, it is unlikely that the detrimental health effects documented in nestlings reared on reclaimed sites (chapters 2, 3 and 4) were due to naphthenic acids. Rather, the birds were probably affected by exposure to other chemicals such as polycyclic aromatic hydrocarbons, by inadequate food supply on reclaimed sites or by other factors associated with the disturbance of various components of ecosystem balance on OSPM sites. Most likely, a combination of all these variables contributed to poorer performance of nestlings on OSPM sites. Nevertheless, it should be kept in mind that in the wild, nestling tree swallows would likely consume NAs from the moment they hatch until the moment they leave reclaimed sites and undertake migration in August, which would represent a total period of approximately 6 to 8 weeks. Findings from the only two studies in which chronic toxicity of NAs was investigated suggest that the effects of longer exposure should be investigated in birds as well. In fish, leucocyte counts only started to decrease 6-10 days after the beginning of exposure to NAs (Dokholyan and Magomedov 1983). In rats, there were no signs of toxicity after one low or medium exposure dose of NAs, but chronic exposure over 90 days resulted in hypocholesterolemia and abnormal accumulation of glycogen in the liver at dosages just 10 times the “worst-exposure” scenario (Rogers et al., 2002a). Rogers’ study also demonstrated that the toxicity of NAs was considerably more dramatic in pregnant female rats than in young, growing males (Rogers, 2003). Perhaps young tree swallows that are still in the nestling stage are relatively resistant to toxic insults and investigating NA toxicity during different developmental stages would have produced significant effects. Measuring hatching success on OSPM sites is not sufficient to discount the hypothesis that NAs do not affect fertility and egg production. In Rogers’s (2003) study, only one of fourteen females actually produced a litter. If on OSPM sites some female tree swallows did not nest because they did not produce any eggs as a result of toxicity, it would go completely undetected because we only monitored nesting females. Thus, experimental exposure studies should be conducted with captive birds in a lab setting to complete our research, so other factors of variation could be controlled and so birds could be closely monitored for an entire generation.

Research should also be conducted with other types of birds because susceptibility to toxicants can be highly species specific. For instance, the egg-shell thinning effects of DDT were not initially detected in the laboratory because testing had been conducted with chickens and quails which are tolerant to DDT (Blus and Henny, 1997). More recently, it has been recognized that vultures of the Indian sub-continent are dying at an alarming rate from intoxication with the veterinary anti-inflammatory drug Diclofenac, to which they are much more sensitive than mammals (Oaks et al., 2004). Although the relative sensitivity of tree swallows to toxicants has never been systematically compared to that of other birds and sensitivity would likely vary from one chemical to another, the lack of overt detrimental effects on survival, reproduction or health in a number studies in which tree swallows were exposed to significant levels of contaminants (Elliot et al., 1994, Custer et al., 1998, Bishop et al., 1999, USGS, 2005) suggests that this species may be quite resilient to toxic insults.

5.6 CONCLUSIONS

Exposure to naphthenic acids for seven days at a dose representing a ten time "worst-exposure scenario" did not result in overt toxicopathological effects in nestling tree swallows. Based on this study, subacute to subchronic toxicity of NAs to nestling tree swallows is minimal. The detrimental health effects documented in nestlings reared on reclaimed sites (chapters 2, 3 and 4) were either due to exposure to other chemicals present in OSPM (such as PAHs), to inadequate food supply on reclaimed sites, to the disturbance of various components of ecosystem balance on OSPM sites, or to a combination of all these factors, rather than to exposure to NAs. However, this study did not investigate chronic or reproductive toxicity of naphthenic acids, which has been previously documented in mammals. More research still needs to be conducted as a part of an assessment of the sustainability of wet landscape reclamation because on reclaimed sites birds will be exposed for weeks to months, and exposure will spawn over the entire breeding period. More research is also needed to characterize the uptake, metabolism and elimination of ingested NAs in higher vertebrates.

CHAPTER 6

6.1 GENERAL DISCUSSION

Oil sands mining companies in Alberta, Canada, are planning reclamation strategies in which wetlands will be used for the bioremediation of water and sediments produced by the oil sand extraction process. This involves the transfer of mine tailings into shallow ponds, where indigenous microbial populations are expected to degrade contaminants. To assess the feasibility of these plans, a series of experimental wetlands and test ponds mimicking future reclaimed habitats were constructed and filled with mine tailings on the two major companies' leases. The first part of this research consists of an assessment of the health of tree swallows (*Tachycineta bicolor*) nesting on those experimental wetlands, where they are exposed to a mixture of chemicals from Oil Sands Process Materials (OSPM) which contain naphthenic acids (NAs) and polycyclic aromatic hydrocarbons (PAHs) as well as other organic and inorganic compounds. The objective was to use endpoints of tree swallow health to evaluate the sustainability of wet landscape reclamation for upper trophic level avian species. Our study sites represented different stages of OSPM bioremediation, from "newly reclaimed" (CT site, which was receiving fresh OSPW during the course of the study) to "mature" (> 10 year old Demo Pond). Suncor's NW could be considered as "intermediate", or "evolving": fresh OSPW was pumped into the wetlands until 2001, leaving two years for microbial communities to degrade contaminants before the beginning of the study (summer 2003). Concentrations of NAs and PAHs in water and sediments varied accordingly to OSPM maturation, being highest in CT, closely followed by NW, and being lowest in Demo Pond. Thus, if bioremediation (on which wet landscape reclamation relies) were progressing as anticipated by the industry, we would expect negative impacts on tree swallow health to be lower on NW than on CT, and to be minimal on Demo Pond.

The second part of this project was specifically aimed at investigating the toxicity of naphthenic acids (NAs) to a native avian species. Naphthenic acids are a complex group of carboxylic acids with surfactant-like properties that are naturally present in petroleum (Schramm et al., 2000). They are a main concern to the oil sands industry because they become highly concentrated in tailings and are responsible for most of the toxicity of process-affected water to aquatic organisms (Leung et al., 2003, van den Heuvel et al., 2000, Pollet and Young, 2000). Information on toxicity of naphthenic acids to terrestrial vertebrates is scarce, as research has been mostly limited to acute exposure studies to establish LD_{50} for laboratory rodents. Only one study investigated chronic toxicity of NAs to mammals. Rogers (2003) found that a dosage only 10 times greater than the highest expected environmental exposure on reclaimed wetlands was sufficient to alter hepatic function and reproduction in rats. Dosing with 60 mg/kg/day NAs over a 90 day period resulted in excessive glycogen accumulation in the liver, elevated plasma amylase and depressed plasma cholesterol. The same dosage administered to females during the breeding period (7 weeks) impaired embryonic implantation, drastically reducing reproductive success: only 7% of females (1 of 14) produced a litter, compared to 100% and 93% in the low dose and control groups, respectively. Toxicity of NAs to birds has never been investigated. To address this need for information, an experimental exposure of tree swallow nestlings to NAs was conducted in 2004 on the reference site Poplar Creek (PC). Nestlings were orally dosed with 0.1 ml of a 15g/L aqueous solution of naphthenic acids from 6 to 13 days of age (approximately one third of their entire post-hatching nestling period) while they were otherwise reared normally by their free-ranging parents.

A total of 53 and 50 breeding pairs nested on the study sites in 2003 and 2004, respectively. Reproductive success was extremely poor on OSPM-sites during the rainy and cold summer of 2003 and fledging success (nestlings fledged/ nestlings hatched) was among the lowest ever reported for a passerine species nesting on sites affected by industrial activities. However, reproduction was relatively unaffected in 2004, when meteorological conditions were less challenging. The poor reproductive performance of 2003 was linked to a widespread nestling die-off which occurred during an episode of harsh weather. During this die-off, mortality rates of nestlings reached 100% and 89% on the two newest reclaimed sites (CT and NW wetlands, respectively), whereas they did not surpass 50% on the reference site. In 2004, mortality rates were low but nestlings reared on OSPM-sites had lower body weight and showed greater EROD activity compared to those on PC. Nestlings on OSPM-sites suffered parasitic burdens (*Protocalliphora sp.* larvae) approximately twice that of those on Poplar Creek (PC). Among birds with comparable parasitic burdens, nestlings on OSPM-sites showed greater detrimental effects (decreased growth) from the infestation than did those on PC. Nestlings from OSPM-sites also exhibited higher levels of thyroid hormones in the blood and in the thyroid glands compared to those from PC.

The harsh weather of 2003 initially gave the project the appearance of a disaster, because more than 2/3 of the nestlings died, greatly reducing sample size and making the second part of the research (experimental exposure to NAs) nearly impossible. Thorough analysis of the data however revealed important and overt effects on population dynamics of tree swallows that had never been documented before on the oil sands. The tree swallow colony on NW has been monitored since 1994 (Paquin, 1994) and those on PC and DP were established in 1997 (Smits et al., 2000). Reproductive performance has been consistently poorer on NW than on other sites (Smits et al., 2000), but was never clearly compromised. Mass mortality of nestlings had never been reported on these reclaimed wetlands (except for mortality related to predators). It is difficult to determine if survival of nestlings on OSPM was low as a direct result of exposure to PAHs and NAs or if it was indirect, i.e., related to limited food supply on OSPM sites. If suitable prey species were just barely abundant enough on OSPM-sites to support tree swallow populations, there might not be any overt effects on nestlings during summers with unchallenging meteorological conditions, but during harsh weather though, a large proportion of insects may be grounded by wind and rain, making them unavailable for foraging (McCarty and Winkler, 1999a). Starving nestlings may not have the metabolic resources necessary to maintain homeothermia during episodes of cold weather. Nevertheless, our data indicate that contaminant exposure cannot completely be discounted because hepatic EROD activity showed a dose-response relationship (i.e., it was highest on CT, then on NW, and so on) similar to that of mortality rates. Mortality rates were highest on CT site (which had the highest concentrations of NAs and presumably PAHs), second highest on NW, 3rd highest on DP and lowest on the reference site, i.e., they decreased accordingly with the concentrations of NAs and PAHs in water and sediments. Furthermore, if food availability had been the main factor limiting nestling survival, mortality rates would have been expected to be highest on Demo Pond, because this site does not support a robust population of benthic invertebrates (Whelly, 1999). The data therefore suggest that the combined exposure to harsh weather and oil sands contaminants precipitated nestling mortality in 2003. The absence of nestling mortality in 2004 demonstrates that unless nestlings were subjected to a combination of stressors, they were relatively resilient to chemical exposure.

One objective of this project was to determine if the wet landscape reclamation strategy planned by the oil sands industry would be sustainable for avian species. Based on endpoints such as EROD activity and nestling survival, our results demonstrate that the wet landscape reclamation strategy has been successful in decreasing the toxicity of OSPM to birds, because mortality rates as well as EROD activity of nestlings reared on ten year old Demo Pond were very close to what we documented on the reference site. However, our findings also indicate that bioremediation takes longer than the 1-2 year period anticipated by the industry (MacKinnon and Boerger, 1986) because mortality rates and EROD were still high on NW, which had not received any fresh OSPM or OSPW (beside dyke seepage) for 2-3 years at the time of our study. Based on these results, it may take as much as ten years (DP is 10 years old) before birds can live on reclaimed sites without suffering detrimental effects in periods of additional stress.

Nestlings on OSPM-sites were smaller than those on Poplar Creek. The decrease in nestling growth on OSPM sites was not as clearly associated with concentrations of

Oil Sands chemicals as were mortality rates and EROD activity. For example, nestlings on NW were consistently the smallest although NAs and PAHs levels were not as high on this site as on neighboring CT wetlands. This suggests that other environmental factors are affecting nestlings along with contaminants. The confounding effect of food availability was not specifically investigated in this project. We used the results from studies measuring the benthic invertebrate biomass to estimate abundance of emergent insects (the main prey of tree swallows), but this method it is not very precise. It would be valuable to include an objective measure of food intake in future studies. Smits et al. (2000) also found nestlings on NW to be smaller than those on other sites, indicating a well-established trend that could have important consequences for nestlings regardless of its cause. Post-fledgling survival is strongly correlated with body mass at fledging (Tinbergen and Boerlijst, 1990, Ringsby et al., 1998, Naef-Daenzer et al., 2001), and young (tree swallows and other bird species) from reclaimed sites may suffer greater post-fledging mortality as a result of their smaller size. The fact that nestling growth was impaired on Demo Pond is of concern because it indicates that based on this endpoint, exposure to OSPM remains detrimental for birds even after ten years of bioremediation.

Larvae of the blowfly *Protocalliphora* spp. (Diptera: Calliphoridae) are obligate hematophagous parasites of altricial birds. They live in nest material and feed intermittently on nestlings' blood until pupation. Although detrimental effects on host health are not always detected, anemia, reduced hemoglobin content within red blood cells and decreased growth are occasionally documented (Whitworth and Bennett, 1992, O'Brien et al., 2001, Morrison and Johnson, 2002). Prevalence of nest infestation with *Protocalliphora* larvae was unexpectedly high on our study sites and surpassed what has been documented for tree swallows in previous research (Rogers et al., 1991, Bennett and Whitworth, 1992, Roby et al., 1992, Thomas and Shutler, 2001, Dawson et al., 2005). We suspect that ongoing mining activities cause a substantial disturbance of several components of the local ecosystem and alter the balance of this host-parasite relationship. For instance, exposure to PAHs and NAs may be compromising nestling immunocompetence, resulting in increased growth, survival and fecundity of their ectoparasites (Bowles et al., 1996, Pruett, 1999, Walker et al., 2003), hematophageous blowfly larvae included. This could eventually lead to the development of large blowfly populations on OSPM sites. Open pit mining involves the removal of old growth forest which decreases the availability of natural cavities for nesting birds, where blowflies lay their eggs. This could result in higher infestation of nest boxes by blowflies. Mining might also affect populations of jewel wasps *Nasonia* spp., which feed on blowfly pupae and kill them, possibly limiting blowfly abundance (Davis et al., 1994, Peters and Abraham, 2004). Well-balanced host-parasite relationships are integral components of healthy ecosystems, and this evidence of an imbalance host-parasite relationship raises questions about the long-term sustainability of wet landscape reclamation.

In avian species, a number of indicators of thyroid function serve as powerful tools for assessing environmental health and can be used to detect endocrine disruption caused by contaminants (Scanes and McNabb, 2003, Mayne et al., 2005). There was an excessive stimulation of thyroid function in nestlings from OSPM sites compared to those from the reference site. Similarly to what was documented for nestling growth, the increase in thyroid hormones levels was not always directly proportional to concentrations of NAs and PAHs in water and sediments of the study sites, i.e., triiodothyronine (T_3) and thyroxine (T_4) in the glands and in plasma were not consistently highest on the most contaminated site. This suggested that other confounding factors such as food availability (reviewed by Sharp and Klandorf, 1985) may also be affecting thyroid function. From all the endpoints measured, thyroid function is the one that suggests the most strongly that the sustainability of current strategies of wet landscape reclamation is still questionable. Thyroid hormones regulate a wide range of physiological processes, including normal metabolism, thermoregulation, growth, molt, as well as mating and breeding behavior (Lothrop, 1996; McNabb, 2000, Colborn, 2002). It is possible that birds reared on OSPM sites would not molt normally because of altered thyroid function, which could affect their chances of migrating successfully, and they might be less likely to survive fall cold spells if their metabolic and thermogenic capacities were compromised as a result of abnormal levels of thyroid hormones. Considering that nestlings reared on OSPM sites are also likely to be smaller and to be recovering from the blood loss inflicted by their heavy parasitic load (chapters 2 and 3), the chances that they would be recruited (i.e., survive to breed successfully) into the population may be quite low. Although logistically challenging, it would be very valuable to extend our monitoring to the post-fledgling period. Evidence from this research predicts that nestlings reared on OSPM-sites would have greater juvenile mortality than nestlings reared on sites unaffected by oil sands mining.

The second part of this study investigated the effects of experimental exposure of nestlings to naphthenic acids. There were no associations between treatment group and growth, hematocrit, blood biochemistry, organ weights or EROD activity. Compared to control nestlings, those exposed to naphthenic acids and saline showed a significant increase in total blood proteins, but this was likely for physiological reasons (dehydration) rather than from toxicity. Histopathological evaluation of major organs, which included a detailed examination of the liver to quantify and qualify hepatocellular vacuolation, did not detect any changes related to NA exposure except for an increase in extramedullary erythropoieis. This finding is important because of its implications for oxygen-carrying capacity of the blood.

The results from our study are comparable to those from similar mammalian studies in which the effects of short exposure to NAs were investigated. We found that nestlings tree swallows were able to tolerate daily exposures to 1.5 mg NAs without major detrimental effects on health, which approximately represents a dose of 0.075 g/kg for a 20 g nestling. Considering that the LD_{50} for laboratory rodents ranges from 3 to 3.55 g/kg (Pennisi and Lynch, 1977, Lewis, 2000) and that Rogers found no toxic response in rats acutely exposed to dosages below 0.3 g/kg, it was to be expected that a dosage of 0.075g/kg would elicit minimal or no reactions in birds (if NAs were metabolized following similar pathways in both vertebrate classes). This dosage was chosen to reflect environmentally realistic concentrations of NAs. It is very possible that exposure to higher doses of NAs would have altered nestling’s health; however, it would be difficult to extrapolate the findings from such a study to oil sands mining reclamation scenarios. It would also be physically impossible to investigate the toxicity of larger doses of NAs to nestling tree swallows. Naphthenic acids cannot be maintained in solution at high concentrations (they precipitate), and we found in a pilot study that larger volumes of dosing solution induced osmotic diarrhea in nestlings.

The results from this study indicate that subacute (7 days) exposure to NAs does not harm nestling tree swallows. Thus, it is unlikely that the detrimental health effects documented in nestlings reared on reclaimed sites (chapters 2, 3 and 4) were due to naphthenic acids. Rather, the birds were probably affected by exposure to other chemicals such as polycyclic aromatic hydrocarbons, or by inadequate food supply on reclaimed sites, or by other factors associated with the disturbance of various components of ecosystem balance on OSPM sites. Most likely, a combination of all these variables contributed to poorer performance of nestlings on OSPM sites. Nevertheless, it should be kept in mind that in the wild, nestling tree swallows would likely consume NAs from the moment they hatch until the moment they leave reclaimed sites and undertake migration in August, which would represent a total period of approximately 6 to 8 weeks. Findings from the only two studies in which chronic toxicity of NAs was investigated suggest that the effects of longer exposure should be investigated in birds as well. In fish, leucocyte counts only started to decrease 6-10 days after the beginning of exposure to NAs (Dokholyan and Magomedov 1983). In rats, there were no signs of toxicity after one low or medium exposure dose of NAs, but chronic exposure over 90 days resulted in hypocholesterolemia and abnormal accumulation of glycogen in the liver at dosages just 10 times the “worst-exposure” scenario (Rogers et al., 2002a). Rogers’ study also demonstrated that the toxicity of NAs was considerably more dramatic in pregnant female rats than in young, growing males (Rogers, 2003). Perhaps young tree swallows that are still in the nestling stage are relatively resistant to toxic insults and investigating NA toxicity during different developmental stages would have produced significant effects. Measuring hatching success on OSPM sites is not sufficient to discount the hypothesis that NAs do not affect fertility and egg production. In Rogers’s (2003) study, only one of fourteen females actually produced a litter. If on OSPM sites some female tree swallows did not nest because they did not produce any eggs as a result of toxicity, it would go completely undetected because we only monitored nesting females. Thus, experimental exposure studies should be conducted with captive birds in a lab setting to complete our research, so other factors of variation could be controlled and so birds could be closely monitored for an entire generation.

CONCLUSIONS

This research provided a broad health assessment of tree swallows inhabiting sites affected by chemicals and materials from Oil Sand mining and extraction. Bioremediation of OSPM appears successful enough to support viable populations of tree swallows on reclaimed sites. However, nestling did not survive on newly reclaimed sites when they were subjected to additional stressors such as harsh weather. They also suffered a number of sub-lethal adverse effects from living on OSPM sites, such as smaller body mass, increased induction of hepatic detoxification enzymes, increased parasitic load and increased thyroid hormones in blood and thyroid glands. This suggests that sustainability of wet landscape reclamation is not optimal yet and may require more improvement. A separate study in which the toxicity of naphthenic acids was investigated revealed that subacute (7 days) exposure could be tolerated by nestling tree swallows without major detrimental effects. These findings suggest that for nestlings reared on oil sands reclaimed sites, exposure to other chemicals such as polycyclic aromatic hydrocarbons and/or inadequate food supply may be a greater concern than exposure to NAs. However, this study did not investigate the chronic or reproductive toxicity of naphthenic acids which has been documented in previous studies with mammals. More research still needs to be conducted as a part of an assessment of the sustainability of wet landscape reclamation because on reclaimed sites birds will be exposed for several weeks and will be breeding.

This research highlights the necessity of conducting long-term research projects to encompass a wide range of meteorological conditions and other natural stressors. Otherwise, the effects of contaminants may be underestimated or go completely undetected. It also emphasizes the value of including new, less traditional endpoints instead of focusing only on the evaluation of growth, reproductive success and hepatic enzyme induction. We also identified the need to extend monitoring to the post-fledging period, which is when nestlings suffer the greatest stress and which has never been monitored on the Oil Sands. This project opened the door to many unresolved questions. It paved the way to further research aiming at elucidating the mechanisms of OSPM toxicity on birds, at characterizing the factors regulating host-parasite interactions on OSPM-sites, and at investigating the effects of long-term, repeated-exposure to naphthenic acids in birds.

BIBLIOGRAPHY

1. Albers, P. H. 2003. Petroleum and individual polycyclic aromatic hydrocarbons. Pages 341-371 *in* D. J. Hoffman, B. A. Rattner, G. A. Burton, and J. Cairns editors. Handbook of Ecotoxicology. CRC Press, Boca Raton, Florida.

2. Alberta Department of Energy. Oil Sands. http://www.energy.gov.ab.ca . 2005.

3. AOSTRA. 1989. AOSTRA (Alberta Oil Sands Technology and Research Authority), Technical Handbook on Oil Sands, Bitumens and Heavy Oils. AOSTRA Edmonton.

4. Asano, H., M. Kobayashi, and T. Hoshino. 1987. The hemopoietic microenvironment in the fetal liver of mice: relationships between developing hepatocytes and erythropblasts. Journal of Electron Microscopy 36:15-25.

5. Beal F.E.L. Food habits of the swallows, a family of valuable native birds. US Department of Agriculture Bulletin 619. 1918.

6. Beckingham J. D. and J. H. Archibald. 1996. Field Guide to Ecosites of Northern Alberta. Canadian Forest Service, Natural Resources of Canada.

7. Bendell-Young, L. I., K. E. Bennett, A. Crowe, C. J. Kennedy, A. R. Kermode, M. M. Moore, A. L. Plant, and A. Wood. 2000. Ecological characteristics of wetlands receiving an industrial effluent. Ecological Applications 10:310-322.

8. Bennett, G. F. 1957. Studies on the genus *Protocalliphora* (Diptera: Calliphoridae). PhD Thesis. University of Toronto.

9. Bennett, G. F., and T. L. Whitworth. 1992. Host, nest, and ecological relationships of species of *Protocalliphora* (Diptera: Calliphoridae). Canadian Journal of Zoology 70:51-61.

10. Bishop, C. A., N. A. Mahony, Trudeau S., and K. E. Pettit. 1999. Reproductive success and biochemical effects in tree swallows (*Tachycineta bicolor*) exposed to chlorinated hydrocarbon contaminants in wetlands of the Great Lakes and St-Lawrence river basin, USA and Canada. Environmental Toxicology and Chemistry 18:263-271.

11. Bishop, C. A., B. Collins, P. Mineau, N. M. Burgess, W. F. Read, and C. Risley. 2000a. Reproduction of cavity-nesting birds in pesticide-sprayed apple orchards in southern Ontario, Canada. Environmental Toxicology and Chemistry 19:588-599.

12. Bishop, C. A., P. Ng, P. Mineau, J. S. Quinn, and J. Struger. 2000b. Effects of pesticide spraying on chick growth, behavior, and parental care in tree swallows (*Tachycineta bicolor*) nesting in an apple orchard in Ontario, Canada. Environmental Toxicology and Chemistry 19:2286-2297.

13. Blus, L. J., and C. J. Henny. 1997. Field studies on pesticides and birds: Unexpected and unique relations. Ecological Applications 7:1125-1132.

14. Boag, B., J. Lello, A. Fenton, D. M. Tompkins, and P. J. Hudson. 2001. Patterns of parasite aggregation in the wild European rabbit (*Oryctolagus cuniculus*). International Journal for Parasitology 31:1421-1428.

15. Boroskova, Z., J. Soltys, and M. Benkova. 1995. Effects of mercury on the immune-response and mean intensity of *Ascaris suum* infection in guinea-pigs. Journal of Helminthology 69:187-194.

16. Bott R. 2005. Canada's oil sands and heavy oil : developing the world's largest petroleum resource. Petroleum Communication Foundation, Calgary.

17. Bowles, V. M., E. N. Meeusen, A. R. Young, A. E. Andrews, A. D. Nash, and M. R. Brandon. 1996. Vaccination of sheep against larvae of the sheep blowfly (*Lucilia cuprina*). Vaccine 14:1347-1352.

18. Brient, J. A., P. J. Wessner, and M. N. Doyle. 1995. Naphthenic acids. Pages 1017-1029 *in* J. I. Kroschwitz editor. Kirk-Othmer Encyclopedia of Chemical Technology. John Wiley and Sons, New York.

19. Brient, J. A. 1998. Commercial utility of naphthenic acids recovered from petroleum distillates. Pages 131-133 *in* The American Chemical Society, Dallas.

20. Brown, C. R., and M. B. Brown. 1986. Ectoparasitsim as a cost of coloniality in cliff swallows (*Hirundo pyrrhonota*). Ecology 67:1206-1218.

21. Brown, C. R., and M. Bomberger Brown. 1998. Intense natural selection on body size and wing and tail asymmetry in cliff swallows during severe weather. Evolution 52:1461-1475.

22. Brown, S. B., B. A. Adams, D. G. Cyr, and J. G. Eales. 2004. Contaminant effects on the teleost fish thyroid. Environmental Toxicology and Chemistry 23:1680-1701.

23. Bryant, D. M. 1978. Environmental influences on growth and survival of nestlings house martins *Delichon urbica.* The Ibis 120:272-283.

24. Burtt, E. H. Jr. 1977. Some factors in the timing of parent chick recognition in swallows. Animal Behavior 25:231-239.

25. Bustnes, J. O., S. A. Hanssen, I. Folstad, K. E. Erikstad, D. Hasselquist, and J. U. Skaare. 2004. Immune function and organochlorine pollutants in arctic breeding glaucous gulls. Archives of Environmental Contamination and Toxicology 47:530-541.

26. Butler, R. W. 1988. Population dynamics and migration routes of Tree Swallows, *Tachycineta bicolor*, in North America. American Journal of Field Ornithology 59:395-402.

27. Cardellini, P., and L. Ometto. 2001. Teratogenic and toxic effects of alcohol ethoxylate and alcohol ethoxy sulfate surfactants on Xenopus laevis embryos and tadpoles. Ecotoxicology and Environmental Safety 48:170-177.

28. Casarett L. J., and J. Doull. 1996. Casarett and Doull's Toxicology: the Basic Science of Poisons., 5th edition. McGraw-Hill, Health Professions Division, New York.

29. CEATAG (CONRAD Environmental Aquatics Technical Adivisory Group). Naphthenic acids background information discussion report. 1998. Edmonton, Alberta Department of Energy

30. Christe, P., A. P. Møller, and F. de Lope. 1998. Immunocompetence and nestling survival in the house martin: the tasty chick hypothesis. Oikos 83:175-179.

31. Christin, M.-S., A. D. Gendron, P. Brousseau, L. Menard, D. J. Marcogliese, D. Cyr, S. Ruby, and M. Fournier. 2003. Effects of agricultural pesticides onthe immune system of *Rana pipiens* and on its resistance to parasitic infection. Environmental Toxicology and Chemistry 22:1127-1133.

32. Clemente, J. S., N. G. N. Prasad, M. D. MacKinnon, and P. M. Fedorak. 2003. A statistical comparison of naphthenic acids characterized by gas chromatography-mass spectrometry. Chemosphere 50:1265-1274.

33. Clemente, J. S. 2004. The characterization, analysis and biodegradation of naphthenic acids. MSc thesis. University of Alberta, Edmonton, Canada.

34. Clemente, J. S., and P. M. Fedorak. 2005. A review of the occurence, analyses, toxicity and biodegradation of naphthenic acids. Chemosphere 60:585-600.

35. Colavecchia, M. V., S. M. Backus, P. V. Hodson, and J. L. Parrott. 2004. Toxicity of Oil Sands to early life stages of fathead minnows (*Pimephales promelas*). Environmental Toxicology and Chemistry 23:1709-1718.

36. Colborn, T. 2002. Clues from wildlife to create an assay for thyroid system disruption. Environ.Health Perspect. 110 Suppl 3 :363-367.

37. Cserhàti, T., E. Forgács, and G. Oros. 2002. Biological activity and environmental impact of anionic surfactants. Environment International 28:337-348.

38. Custer, C. M., T. W. Custer, P. D. Allen, K. L. Stromborg, and M. J. Melancon. 1998. Reproduction and environmental contamination in tree swallows nesting in Fox River drainage and Green Bay, Wisconsin, USA. Environmental Toxicology and Chemistry 17:1786-1798.

39. Custer, C. M., T. W. Custer, P. M. Dummer, and K. Munney. 2003. Exposure and effects of chemicals contaminants on tree swallows nesting along the Housatonic river, Berkshire county, Massachusetts, USA, 1998-2000. Environmental Toxicology and Chemistry 22:1605-1621.

40. Custer, T. W., C. M. Custer, K. Dickerson, K. Allen, M. J. Melancon, and L. J. Schmidt. 2001. Polycyclic aromatic hydrocarbons, aliphatic hydrocarbon, trace elements, and monooxygenase activity in birds nesting on the North Platte river, Casper, Wyoming, USA. Environmental Toxicology and Chemistry 20:624-631.

41. Daoust, P.-Y., G. Conboy, S. McBurney, and N. Burgess. 1998. Interactive mortality factors in common loons from maritime Canada. Journal of Wildlife Diseases 34:524-531.

42. Davis, L. 1992. Case histories - The petroleum refining industry. Pages 183-223 *in* Toxicity Reduction Evaluation and Control. Techonomic Publishing Company, Lancaster, Pennsylvania, USA.

43. Davis, W. H., P. J. Kalisz, and R. J. Wells. 1994. Eastern bluebirds prefer boxes containing old nests. Journal of Field Ornithology 65:250-253.

44. Dawson, A., V. M. King, G. E. Bentley, and G. F. Ball. 2001. Photoperiodic control of seasonality in birds. Journal of Biological Rhythms 16:365-380.

45. Dawson, R. D., K. K. Hillen, and T. L. Whitworth. 2005. Effect of experimental variation in temperature on larval densities of parasitic *Protocalliphora* (Diptera:Calliphoridae) in nests of tree swallows (Passeriformes: Hirundinae). Environmental Entomology 34:563-568.

46. Dohoo, I., W. Martin, and H. Stryhn. 2003. Introduction to clustered data. Pages 460-473 *in* S. M. McPike editor. Veterinary Epidemiologic Research. AVC inc., Charlottetown.

47. Dokholyan, B. K., and A. K. Magomedov. 1983. Effects of sodium naphthenate on survival and on some physiological-biochemical parameters of some fishes. Journal of Ichtyology 23:125-132.

48. duBowy, P. J., and S. W. Moore. 1985. Weather-related mortality in swallows in the Sacramento Valley of California. Western Birds 16:49-50.

49. Dunn, P. O., and S. J. Hannon. 1992. Effects of food abundance and male parental care on reproductive success and monogamy in tree swallows. The Auk 109:488-499.

50. Dwilvedi, P., and R. B. Burns. 1984. Pathology of ochratoxicosis A in young broiler chicks. Research in Veterinary Science 36:92-103.

51. Eeva, T., E. Lehikoinen, and J. Nurmi. 1994. Effects of ectoparasites on breeding success of great tits (*Parus major*) and pied flycatchers (*Ficedula hypoleuca*) in an air pollution gradient. Canadian Journal of Zoology 72:624-635.

52. Eeva, T., and E. Lehikoinen. 1996. Growth and mortality of nestling great tits (*Parus major*) and pied flycatchers (*Ficedula hypoleuca*) in a heavy metal pollution gradient. Oecologia 108:631-639.

53. Elliott, J. E., P. A. Martin, T. W. Arnold, and P. H. Sinclair. 1994. Organochlorines and reproductive success of birds in orchards and non-orchards areas of central British-Columbia, Canada, 1990-1991. Archives of environmental contamination and toxicology 26:435-443.

54. Erskine A.J. 1977. Birds in boreal Canada: communities, densities and adaptations. Report no 41, Canadian Wildlife Service Repositery Series.

55. Fairbrother, A., J. Smits, and K. A. Grasman. 2004. Avian Immunotoxicology. Journal of Toxicology and Environmental Health-Part B-Critical Reviews 7:105-137.

56. Fox, G. A. 2001. Effects of endocrine disrupting chemicals on wildlife in Canada: Past, present and future. Water Quality Research Journal of Canada 36:233-251.

57. FTFC (Fine Tailings Fundamental Consortium). 1995. Fine tails and process water reclamation. Pages 1-52 *in* Advances in Oil Sands Tailings Research. Alberta Department of Energy, Oil Sands Research Division, Edmonton.

58. Galvini, A. P. 2003. Immunity, antigenic heterogeneity, and aggregation of helminth parasites. Journal of Parasitology 89:232-241.

59. Ganshorn, K. D. 2002. Secondary production, trophic position, and potential for accumulation of polycyclic hydrocarbons in predatory diptera in four wetlands of the Athabasca Oil Sands, Alberta, Canada. MSc thesis. University of Windsor, Windsor, Canada.

60. Gerrard, P. M., and V. L. St-Louis. 2001. The effects of experimental reservoir creation on the bioaccumulation of methylmercury and reproductive success of tree swallows (*Tachycineta bicolor*). Environmental Science and Technology 35:1329-1338.

61. Golder Associates Ltd. 1997. Field scale trials to assess effects of consolidated tails release water on plants and wetlands ecology. Report no 962-1881. Calgary.

62. Golder Associates Ltd. 1998. Synthesis of environmental information on consolidated/composite tailings (CT). Report no 972-2205.6045. Calgary.

63. Golder Associates Ltd. 2002. Wetlands study sites associated with Oil sands research and monitoring. Report no. 022-2203. Calgary.

64. Golder Associates Ltd. 2003. Wildlife baseline report for the Suncor South tailings pond project. Report no 03-1322-091. Calgary.

65. Golder Associates Ltd. 2004. Consolidated tailings (CT) integrated reclamation landscape demonstration project. Report no 03-1322-005. Calgary.

66. Grasman, K. A., and G. A. Fox. 2001. Associations between altered immune function and organochlorine contamination in young Caspian terns (*Sterna caspia*) from lake Huron, 1997-1999. Ecotoxicology 10:101-114.

67. Grenfell, B. T., K. Wilson, V. S. Isham, H. E. G. Boyd, and K. Dietz. 1995. Modelling patterns of parasite aggregation in natural populations: Trichostrongylid nematode-ruminant interactions as a case study. Parasitology 111:S135-S151.

68. Gulley, J. R. and M. D. MacKinnon. 1993. Fine tails reclamation utilizing a wet landscape approach. Pages 1-24 *in* AOSTRA, Alberta Chamber of Resources, Energy, Mines and Resources, Edmonton.

69. Gurney, K. E., T. D. Williams, J. E. Smits, M. Wayland, S. Trudeau, and L. I. Bendell-Young. 2005. Impact of Oil-Sands based wetlands on the growth of mallard (*Anas platyrhynchos*) ducklings. Environmental Toxicology and Chemistry 24:457-463.

70. Hammad, E. F., J. S. Stiffler, and R. R. Cardell. 1982. Morphological and biochemical observations on hepatic glycogen metabolism in mice on a controlled feeding shcedule: normal mice. Digestive Diseases and Sciences 27:680-690.

71. Harris, M. L., and J. E. Elliott. 2000. Reproductive success and chlorinated hydrocarbon contamination in tree swallows (*Tachycineta bicolor*) nesting along rivers receiving pulp and paper mill effluent discharges. Environmental Pollution 110:307-320.

72. Hart, B. T., P. Bailey, R. Edwards, K. Hortle, K. James, A. McMahon, C. Merdith, and K. Swadling. 1990. Effects of salinity on river, stream and wetland ecosystems in Victoria, Australia. Water Research 24:1103-1117.

73. Harvey, S., H. Klandorf, and J. G. Phillips. 1981. Reproductive performance and endocrine responses to ingested petroleum in domestic ducks (*Anas platyrhynchos*). General and Comparative Endocrinology 45:372-380.

74. Headley, J. V., K. M. Peru, D. W. McMartin, and M. Winkler. 2002. Determination of dissolved naphthenic acids in natural waters by using negative-ion electrospray mass spectrometry. Journal of AOAC International 85:182-187.

75. Heeb, P., M. Kolliker, and H. Richner. 2000. Bird-ectoparasite interactions, nest humidity and ectoparasite community structure. Ecology 81:958-968.

76. Herman, D. C., P. M. Fedorak, M. D. MacKinnon, and J. W. Costerton. 1994. Biodegradation of naphthenic acids by microbial populations indigenous to oil sands tailings. Canadian Journal of Microbiology 40:467-477.

77. Hill, J. R., and L. Chamber. 1992. Purple Martin weather deaths during the summer of '92. Purple Martin Update 4:6-10.

78. Holowenko, F. M., M. D. MacKinnon, and P. M. Fedorak. 2002. Characterization of naphthenic acids in oil sands wastewaters by gas chromatography-mass spectrometry. Water Research 36:2843-2855.

79. Hox J. J. 2002. Multilevel Analysis: Techniques and Applications. Lawrence Erlbaum Associates, Mahwah, N.J.

80. Hurtrez-Boussès, S., J. Blondel, P. Perret, and F. Renaud. 1997. Relationship between intensity of blowfly infestation and reproductive success in a corsican population of Blue Tits. Journal of Avian Biology 28:267-270.

81. Hurtrez-Boussès, S., M. de Garine-Wichatitsky, P. Perret, and J. Blondel. 1999. Variations in prevalence and intensity of blow fly infestations in an insular Mediterranean population of blue tits. Canadian Journal of Zoology 77:337-341.

82. Hussell, D. J. T. 1982. Longevity and fecundity records in the Tree Swallow. North American Bird Bander 7:154.

83. Hussell, D. J. T. 1983. Age and plumage color in female tree swallow. Journal of Field Ornithology 54:312-318.

84. Jacobs, J. D., and J. C. Wingfield. 2000. Endocrine control of life-cycle stages: A constraint on response to the environment? Condor 102:35-51.

85. Jain N. C. 1986. Schalm's Veterinary Hematology., 4th edition. Lea and Febiger, Philadelphia.

86. Janssens, E., T. Dauwe, R. Pinxten, and M. Eens. 2003. Breeding performance of great tits (*Parus major*) along a gradient of heavy metal pollution. Environmental Toxicology and Chemistry 22:1140-1145.

87. Jenssen, B. M., M. Ekker, and K. Zahlsen. 1990. Effects of ingested crude-oil on thyroid hormones and on the mixed-function oxidase system in ducks. Comparative Biochemistry and Physiology Part C-Pharmacology Toxicology & Endocrinology 95:213-216.

88. Jones, D. M., J. S. Watson, W. Mereditth, M. Chen, and B. Bennett. 2001. Determination of naphthenic acids in crude oils using nonaquous ion exchange solid-phase extraction. Analytical Chemistry 73:703-707.

89. Jones, J. 2003. Tree swallows (*Tachycineta bicolor*): a new model organism? The Auk 120:591-599.

90. Keedwell, R. J. 2003. Does fledging equal success? Post-fledging mortality in the Black-fronted tern. Journal of Field Ornithology 74:217-221.

91. Keith, J. O., and C. A. Mitchell. 1993. Effects of DDE and food stress on reproduction and body condition of ringed turtle doves. Archives of Environmental Contamination and Toxicology 25:192-203.

92. Kershner, E. L., J. W. Walk, and R. E. Warner. 2004. Postfledging movements and survival of juvenile eastern meadowlarks (*Sturnella magna*) in Illinois. The Auk 121:1146-1154.

93. Khanna, Y. P., P. S. Chaudhary, P. Singh, and S. D. Varma. 1972. Physiological effects of naphthenic acid: a new reproductive agent. Indian Journal of Experimental Biology 10:149-150.

94. Krasnov, B. R., I. S. Khokhlova, M. S. Arakelyan, and A. A. Degen. 2005. Is a starving host tastier? Reproduction in fleas parasitizing food-limited rodents. Functional Ecology 19:625-631.

95. Lai, J. W. S., L. J. Pinto, E. Kiehlmann, L. I. Bendell-Young, and M. M. Moore. 1996. Factors that affect the degradation of naphthenic acids in Oil Sands wastewater by

indigenous microbial communities. Environmental Toxicology and Chemistry 15:1482-1491.

96. Lawrence, G. A., P. R. B. Ward, and M. D. MacKinnon. 1991. Wind and wave-induced suspension of mine tailings in disposal ponds -a case study. Canadian Journal of Civil Engineering 18:1047-1053.

97. Lemly, A. D. 1993. Metabolic stress during winter increases the toxicity of selenium to fish. Aquatic Toxicology 27:133-158.

98. Leonhardt, C. L. 2003. Zoobenthic succession in constructed wetlands of the Fort McMurray Oil Sands region: developping a measure of zoobenthic recovery. MSc thesis. University of Windsor, Canada.

99. Leung, S. S., M. D. MacKinnon, and R. E. H. Smith. 2001. Aquatic reclamation in the Athabasca, Canada, Oil Sands: naphthenates and salt effects on phytoplankton communities. Environmental Toxicology and Chemistry 20:1532-1543.

100. Leung, S. S., M. D. MacKinnon, and R. E. H. Smith. 2003. The ecological effects of naphthenic acids and salt on phytoplankton from the Athabasca Oil Sands region. Aquatic Toxicology 62:11-26.

101. Lewis, M. A. 1991. Chronic and sublethal toxicities of surfactants to aquatic animals - A review and risk assessment. Water Research 25:101-113.

102. Lewis R. J. 2000. Sax's Dangerous Properties of Industrial Materials., 10th edition. Wiley-Interscience, New York.

103. Lothrop, C. D. 1996. Diseases of the endocrine system. Pages 368-379 *in* W. J. Rosskopf, and R. W. Woerpel editors. Diseases of Cage and Aviary Birds. Williams and Wilkins, Baltimore, USA.

104. Luebke, R. W., C. B. Copeland, J. J. Diliberto, P. I. Akubue, A. E. Andrews, M. M. Riddle, H. H. Williams, and J. L. Birnbaum. 1994. Assessment of host-resistance to *Trichinella spiralis* in mice following pre infection exposure to 2,3,7,8-TCDD. Toxicology and Applied Pharmacology 125:7-16.

105. Mackay W. & Verbeek A.G. 1993. The characterization of acutely toxic compounds in fine tails pore water and tailings pond water. AOSTRA 8878 (Sludge 681). Edmonton, AOSTRA (Alberta Oil Sands Technology and Research Authority), Alberta Department of Energy.

106. MacKinnon, M. D. and J. T. Retallack. 1982. Preliminary characterization and detoxification of tailings pond water at the Syncrude Canada Ltd. oil sands plant. Pages 185-210 *in* Denver, USA.

107. MacKinnon, M. D., and H. Boerger. 1986. Description of two treatments for detoxifying oil sands tailings pond water. Water Pollution Research Journal of Canada 21:496-512.

108. Madill, R. E. A., M. T. Orzechowski, G. Chen, B. G. Brownlee, and N. J. Bunce. 2001. Preliminary risk assessment of the Wet Landscape Option for reclamation of Oils Sands mine tailings: bioassays with mature fine tailings pore water. Environmental Toxicology 16:197-208.

109. Maizelis M.I., Kruglikov R.I., Gaibov T.D., Omarov I.A. & Zabludovskii A.L. Effect of cyclopentane naphthenic acids and hydrocarbons on hemato-encephalic barrier permeability. Vopr Kurortol Fizioter Lech Fiz Kult March-April[2], 61-63. 1980.

110. Mann, R. M., and J. R. Bidwell. 2001. The acute toxicity of agricultural surfactants to the tadpoles of four Australian and, two exotic frogs. Environmental Pollution 114:195-205.

111. Mayne, G. J., C. A. Bishop, P. A. Martin, H. J. Boermans, and B. Hunter. 2005. Thyroid function in nestling tree swallows and eastern bluebirds exposed to non-persistent pesticides and p,p '-DDE in apple orchards of southern Ontario, Canada. Ecotoxicology 14:381-396.

112. Mcarthur, M. L. B., G. A. Fox, D. B. Peakall, and B. J. R. Philogene. 1983. Ecological significance of behavioral and hormonal abnormalities in breeding ring doves fed an organochlorine chemical mixture. Archives of environmental contamination and toxicology 12:343-353.

113. McCarty, J. P., and D. W. Winkler. 1991. Use of an artificial nestling for determining the diet of nestling tree swallows. Journal of the Field Ornithologist 62:211-217.

114. McCarty, J. P., and A. L. Secord. 1999. Reproductive ecology of tree swallows (*Tachycineta bicolor*) with high levels of polychlorinated biphenyl contamination. Environmental Toxicology and Chemistry 18:1433-1439.

115. McCarty, J. P., and D. W. Winkler. 1999a. Relative importance of environmental variables in determining the growth of nestling Tree Swallows *Tachycineta bicolor*. Ibis 141:286-296.

116. McCarty, J. P., and D. W. Winkler. 1999b. Foraging ecology and diet selectivity of tree swallows feeding nestlings. Condor 101:246-254.

117. McCarty, J. P. 2002. The number of visits to the nest by parents is an accurate measure of food delivered to nestlings in Tree Swallows. Journal of the Field Ornithologist 73:9-14.

118. McNabb, F. M. A., and M.-F. Cheng. 1985. Thyroid development in Ring Doves, *Streptopelia risoria.* General and Comparative Endocrinology 58:243-251.

119. McNabb, F. M. A., and D. B. King. 1993. Thyroid hormone effects on growth, development and metabolism. Pages 393-417 *in* M. P. Schreibman, C. G. Scanes, and P. K. T. Pang editors. The Endocrinology of Growth, Development, and Metabolism of Vertebrates. Academic Press, New York.

120. McNabb, F. M. A. 2000. Thyroids. Pages 461-471 *in* G. C. Whittow editor. Sturkie's Avian Physiology. Academic Press, San Diego.

121. McNabb, F. M. A. 2005. Biomarkers for the assessment of avian thyroid disruption by chemical contaminants. Avian and Poultry Biology Reviews 16:3-10.

122. Mikula, R. J., K. L. Kasperski, R. Burns, and M. D. MacKinnon. 1996. The nature and fate of oil sands fine tailings. Pages 677-723 *in* L. L. Schramm editor. Suspensions: Fundamentals and Applications in the Petroleum Industry. American Chemical Society, Washington D.C.

123. Moles, A., and T. L. Wade. 2001. Parasitism and phagocytic function among sand lance *Ammodytes hexapterus* pallas exposed to crude oil-laden sediments. Bulletin of Environmental Contamination and Toxicology 66:528-535.

124. Morrisson, B. L., and L. S. Johnson. 2002. Feeding of house wren nestlings afflicted by hematophagous ectoparasites: a test of the parental compensation hypothesis. The Condor 104:183-187.

125. Murray L.J., Arner B.D. & Pauls R.W. 1988. Breeding woodland birds near the Syncrude Canada Ltd. Oil Sands plant, 1986. Report for Syncrude Canada ltd.

126. Naef-Daenzer, B., F. Widmer, and M. Nuber. 2001. Differential post-fledging survival of great and coal tits in relation to their condition and fledging date. Journal of Animal Ecology 70:730-738.

127. Nayak, N. C., A. S. Sitara, S. M. S. Duttagupta, M. Mathur, and P. Chopra. 1996. The nature and significance of liver cell vacuolation following hepatocellular injury - an analysis based on observations on rats rendered tolerant to hepatotoxic damage. Virchows Archives 428:353-365.

128. Neff, J. M. 1985. Polycyclic aromatic hydrocarbons. Page -666 *in* G. M. Rand, and S. R. Petrocelli editors. Fundamentals of Aquatic Toxicology : Methods and Applications. Hemisphere, New York.

129. Nix P.G., Hamilton S.H., Bauer E.D. & Gunter C.P. 1993. Constructed wetlands for the treatment of oil sands wastewater. Edmonton, Canada, AOSTRA (Alberta Oil Sands Technology and Research Authority).

130. O'Brien, E. L., B. L. Morrison, and L. S. Johnson. 2001. Assessing the effects of haematophagous ectoparasites on the health of nesting birds: haematocrit vs haemoglobin levels in House Wrens parasitized by blow fly larvae. Journal of Avian Biology 32:73-76.

131. Oaks, J. L., M. Gilbert, M. Z. Virani, R. T. Watson, C. U. Meteyer, B. A. Rideout, H. L. Shivaprasad, S. Ahmed, C. Iqbal, J. Muhammad, M. Arshad, S. Mahmood, A. Ali, and A. Ahmed Khan. 2004. Diclofenac residues as the cause of vulture population decline in Pakistan. Nature 427:630-633.

132. Oetken, M., J. Bachmann, U. Schulte-Oehlmann, and J. Oehlmann. 2004. Evidence for endocrine disruption in invertebrates. International Review of Cytology - A Survey of Cell Biology 1-44.

133. OSERN (Oil Sands Environmental Research Network), University of Alberta. 2004. Frequently Asked Questions About the Oil Sands. http://www.osern.rr.ualberta.ca/index.

134. Paquin L. 1994. Wetlands tree swallow bird box study. Report no 1307, Suncor Inc.

135. Parrott J.L., Hodson P.V., Tillitt D.E., Bennie D.T. & Comba M.E. 1996. Accumulation of fish mixed-function oxygenase inducers by semi-permeable membrane devices in river water and effluents, Athabasca River, August and September, 1994. Report 83, Project 2345-D1. Edmonton, Canada. Northern River Basins Study Project.

136. Patrick, R., J. Cairns, and A. Scheier. 1968. The relative sensitivity of diatoms, snails, and fish to twenty common constituents of industrial wastes. The Progressive Fish Culturist 30:137-140.

137. Peakall, D. B., J. Tremblay, W. B. Kinter, and D. S. Miller. 1981. Endocrine dysfunction in seabirds caused by ingested oil. Environmental Research 24:6-14.

138. Pennisi S.C. & dePaul Lynch V. 1977. Acute and sub-acute toxicity of naphthenic acids. The Pharmacologist 19[181], 208. Abstract

139. Peters, L. E. 1999. The effects of oil sands aquatic reclamation on the early-life stages of fish. MSc thesis. University of Waterloo, Waterloo, Ontario, Canada.

140. Peters, R., and R. Abraham. 2004. Interactions between hymenoptera (Chalcidoidea:Pteromalidae) and diptera: Cyclorrhapha in nests of cavity-nesting birds. Entomologia Generalis 27:133-142.

141. Petrie A., and P. Watson. 1999. Statistics for Veterinary and Animal Science. Blackwell Science Ltd., Oxford, UK.

142. Pollet, I., and L. I. Bendell-Young. 2000. Amphibians as indicators of wetland quality in wetlands formed from Oil Sands effluents. Environmental Toxicology and Chemistry 19:2589-2597.

143. Pruett, J. H. 1999. Immunological control of arthropod ectoparasites: a review. International Journal of Parasitology 29:25-32.

144. Puchala, P. 2004. Detrimental effects of larval blow flies (*Protocalliphora azurea*) on nestlings and breeding success of Tree Sparrows (*Passer montanus*). Canadian Journal of Zoology 82:1285-1290.

145. Quagraine, E. K., H. G. Peterson, and J. V. Headley. 2005. In situ bioremediation of naphthenic acids contaminated tailing pond waters in the Athabasca Oil Sands region-demonstrated field studies and plausible options: a review. Journal of Environmental Science and Health 40:685-722.

146. Rae, M. 2000. Avian endocrine disorders. *in* A. M. Fudge editor. Laboratory Medicine: Avian and Exotic Pets. WB Saunders Co, Philadelphia.

147. Rendell, W. B., and N. A. M. Verbeek. 1996a. Old nest material in nest boxes of tree swallows: effects on reproductive success. The Condor 98:142-152.

148. Rendell, W. B., and N. A. M. Verbeek. 1996b. Are avian ectoparasites more numerous in nest boxes with old nest material? Canadian Journal of Zoology 74:1819-1825.

149. Ringsby, T. H., B.-E. Sæther, and J. Solberg. 1998. Factors affecting juvenile survival in House Sparrow *Passer domesticus*. Journal of Avian Biology 29:241-247.

150. Robertson, R. J., B. J. Stutchbury, and R. R. Cohen. 1992. Tree Swallow. Pages 1-27 *in* A. Poole, P. Stettenheim, and K. Kaufman editors. The Birds of North America. The American Ornithologists' Union & The Academia of Natural Sciences, Philadelphia, PA.

151. Robertson, R. J., and W. B. Rendell. 2001. A long-term study of reproductive performance in tree swallows: the influence of age and senescence on output. Journal of Animal Ecology 70:1014-1031.

152. Roby, D. D., K. L. Brink, and K. Wittmann. 1992. Effects of bird blowfly parasitism on Eastern Bluebird and Tree Swallow nestlings. Wilson Bulletin 104:630-643.

153. Rogers, C. A., J. Raleigh, R. J. Robertson, and B. J. Stutchbury. 1991. Patterns and effects of parasitism by *Protocalliphora sialia* on tree swallow nestlings. Pages 123-139 *in* J. E. Loye, and M. Zuk editors. Bird-Parasite Interactions. Oxford University Press, Oxford.

154. Rogers, V. V., M. Wickstrom, K. Liber, and M. D. MacKinnon. 2002a. Acute and subchronic mammalian toxicity of naphthenic acids from Oil Sands tailings. Toxicological Sciences 66:347-355.

155. Rogers, V. V., K. Liber, and M. D. MacKinnon. 2002b. Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. Chemosphere 48:519-527.

156. Rogers, V. V. 2003. Mammalian toxicity of naphthenic acids derived from the Athabasca Oil Sands. PhD thesis. University of Saskatchewan, Saskatoon, SK, Canada.

157. Rolland, R. M. 2000. A review of chemically-induced alterations in thyroid and vitamin A status from field studies of wildlife and fish. Journal of Wildlife Diseases 36:615-635.

158. Rudzinski, W. E., L. Oehlers, and Y. Zhang. 2002. Tandem mass spectrometric characterization of commercial naphthenic acids and a Maya crude oil. Energy Fuels 16:1178-1185.

159. Sabrosky C. W., G. F. Bennett, and T. L. Whitworth. 1989. Bird blow flies (*Protocalliphora*) in North America (Diptera:*Calliphoridae*) with notes on Palearctic species., 1st edition. The Smithsonian Institution Press, Washington.

160. Sagerup, K., E. O. Henriksen, A. Skorping, J. U. Skaare, and G. W. Gabrielson. 2000. Intensity of parasitic nematodes increases with organochlorine levels in glaucous gull. Journal of Applied Ecology 37:532-539.

161. Scanes, C. G., and F. M. A. McNabb. 2003. Avian models for research in toxicology and endocrine disruption. Avian and Poultry Biology Reviews 14:21-52.

162. Schramm, L. L., E. N. Stasiuk, and M. D. MacKinnon. 2000. Surfactants in Athabasca Oil Sands slurry conditioning, flotation recovery, and tailings processes. Chapter 10 *in* L. L. Schramm editor. Surfactants : fundamentals and applications in the petroleum industry. Cambridge University Press, Cambridge, NY, USA.

163. Sharp, P. J., and H. Klandorf. 1985. Environmental and physiological factors controlling thyroid function in Galliformes. Pages 175-188 *in* B. K. Follett, S. Ishii, and A. Chandola editors. The Endocrine System and the Environment. Japan Scientific Societies Press and Springer-Verlag, Tokyo, Berlin.

164. Shield, W. M., and J. R. Crook. 1987. Barn swallow coloniality: a net cost for group breeding in the Adirondacks? Ecology 68 :1373-1386.

165. Shortt R. 1988. Bird use of alternate waterbodies in the Syncrude Canada Ltd.area, 1985. Report for Syncrude Wildlife Protection Division.

166. Shutler, D., and R. G. Clark. 2003. Causes and consequences of tree swallow (*Tachycineta bicolor)* dispersal in Saskatchewan. Auk 120:619-631.

167. Sik-Cheung Leung, S., M. D. MacKinnon, and R. E. H. Smith. 2001. Aquatic reclamation in tha Athabasca, Canada, Oil Sands: naphthenate and salt effects on phytoplankton communities. Environmental Toxicology and Chemistry 20:1532-1543.

168. Silva, J. E. 1995. Thyroid hormone control of thermogenesis and energy balance. Thyroid 5:481-492.

169. Simon, A., D. W. Thomas, M. M. Blondel, M. M. Lambrechts, and P. Perret. 2003. Within-brood distribution of ectoparasite attacks on nestling blue tits: a test of the tasty chick hypothesis using insulin as a tracer. Oikos 102:551-558.

170. Simon, A., D. Thomas, J. Blondel, P. Perret, and M. M. Lambrechts. 2004. Physiological ecology of Mediterranean Blue Tits (*Parus caeruleus L.*): Effects of ectoparasites (*Protocalliphora spp.*) and food abundance on metabolic capacity of nestlings. Physiological and Biochemical Zoology 77:492-501.

171. Singh, H. 1989. Interaction of xenobiotics with reproductive endocrine functions in a protogynous teleost, *Monopterus Albus.* Marine Environmental Research 28:285-289.

172. Siwik, P. L., T. Van Meer, M. D. MacKinnon, and C. Paszkowki. 2000. Growth of fathead minnows in Oil Sands-Process wastewater in laboratory and field. Environmental Toxicology and Chemistry 19:1837-1845.

173. Smits, J. E., and T. D. Williams. 1999. Validation of immunotoxicology techniques in passerine chicks exposed to Oil Sands tailings water. Ecotoxicology and Environmental Safety 44:105-112.

174. Smits, J. E., M. E. Wayland, M. J. Miller, K. Liber, and S. Trudeau. 2000. Reproductive, immune and physiological endpoints in tree swallows on reclaimed Oil Sands mine sites. Environmental Toxicology and Chemistry 19:2951-2960.

175. Stephens, S. M., A. Y. A. Alkindi, C. P. Waring, and J. A. Brown. 1997. Corticosteroid and thyroid responses of larval and juvenile turbot exposed to the water-soluble fraction of crude oil. Journal of Fish Biology 50:953-964.

176. Strausz O. P., and E. M. Lown. 2003. The chemistry of Alberta Oil Sands, bitumen and heavy oils. Alberta Energy Research Institute, Calgary, Canada.

177. Stutchbury, B. J., and R. J. Robertson. 1988. Within-season and age-related patterns of reproductive performance in female tree swallows (*Tachycineta bicolor*). Canadian Journal of Zoology 66:827-834.

178. Syncrude Canada Ltd. 2005. Frequently Asked Questions About Syncrude. http://www.syncrude.com/who_we_are

179. Tetreault, G. R., M. E. McMaster, D. G. Dixon, and J. L. Parrott. 2003. Using reproductive endpoints in small forage fish species to evaluate the effects of Athabasca Oil Sands activities. Environmental Toxicology and Chemistry 22:2775-2782.

180. Thomas, K., and D. Shutler. 2001. Ectoparasites, nestling growth, parental feeding rates, and begging intensity of tree swallows. Canadian Journal of Zoology 79:346-353.

181. Thophona, S., M. Kruatrachue, E. S. Upathama, S. Pokethitiyooka, S. Sahaphongb, and S. Jaritkhuanc. 2003. Histological alterations of white seabass, *Lates calcarifer*, in acute and subchronic cadmium exposure. Environmental Pollution 121:307-320.

182. Tinbergen, J. M., and M. C. Boerlijst. 1990. Nestling weight and survival in individual great tits (*Parus major*). Journal of Animal Ecology 59:1113-1127.

183. Trudeau S. & Maisonneuve F.J. 2001. A method to determine cytochrome P4501A activity in wildlife microsomes. Report no 339E, Hull, QC, Canadian Wildlife Service, Headquarters. Technical Report Series.

184. US Geological Survey (USGS) Patuxent Wildlife Research Center. 2005. Biological and ecotoxicological characteristics of terrestrial vertebrate species residing in estuaries: tree swallow. http://www.pwrc.usgs.gov/bioeco/treeswal.html.

185. van den Heuvel, M. R., M. Power, M. D. MacKinnon, T. Van Meer, E. P. Dobson, and D. G. Dixon. 1999a. Effects of oil sands related aquatic reclamation on yellow perch (*Perca flavescens*). I. water quality characteristics and yellow perch physiological and population responses. Canadian Journal of fisheries and Aquatic Sciences 56:1213.

186. van den Heuvel, M. R., M. Power, M. D. MacKinnon, and D. G. Dixon. 1999b. Effects of oil sands related aquatic reclamation on yellow perch (*Perca flascens*). II. Chemical and biochemical indicators of exposure to oil sands related water. Canadian Journal of fisheries and Aquatic Sciences 56:1226-1233.

187. van den Heuvel, M. R., M. Power, J. Richards, M. D. MacKinnon, and D. G. Dixon. 2000. Disease and gill lesions in yellow perch (*Perca flavescens*) exposed to Oil Sands mining-associated waters. Ecotoxicology and Environmental Safety 46:334-341.

188. Visser, G. H. 1998. Development of temperature regulation. Pages 117-156 *in* M. Starck, and R. E. Ricklefs editors. Avian Growth and Development: Evolution Within the Altricial-Precocial Spectrum. Oxford University Press, Cary, NC, USA.

189. Voudrias, E. A., and C. L. Smith. 1986. Hydrocarbon pollution from marinas in estuarine sediments. Estuarine Coastal Shelf Science 22:272-284.

190. Wakelin D. 1996. Immunity to Parasites: How Parasitic Infections Are Controlled., 2nd edition. Cambridge University Press, Cambridge, UK.

191. Walker, M., S. Steiner, M. W. G. Brinkhof, and H. Richner. 2003. Induced responses of nestlings great tits reduce hen flea reproduction. Oikos 102:67-74.

192. Wayland, M., S. Trudeau, T. Marchant, D. Parker, and K. A. Hobson. 1998. The effect of pulp and paper mill effluent on an insectivorous bird, the tree swallow. Ecotoxicology 7:237-251.

193. Wayland, M., H. G. Gilchrist, D. L. Dickson, T. Bollinger, C. James, R. A. Carreno, and J. Keating. 2001. Trace elements in king eiders and common eiders in the Canadian Arctic. Archives of Environmental Contamination and Toxicology 41:491-500.

194. Wayland M. & Smits J.E. 2003. The ecological viability of constructed wetlands at Suncor: population and health-related considerations in birds. Environment Canada. Assessment of natural and anthropogenic impacts of oil sands contaminants within the northern river basins. Final summary report -Task 5: hydrocarbons/oil sands and heavy oil research and development.

195. Wesolowski, T. 2001. Host-parasite interactions in natural holes: marsh tits (*Parus palustris*) and blow flies. The Zoological Society of London 255:495-503.

196. Whelly, M. P. 1999. Aquatic invertebrates in wetlands of the Oil Sands region of northeast Alberta, Canada, with emphasis on Chironomidae (Diptera). MSc thesis. University of Windsor, Canada.

197. Whitworth, T. L. 1976. Host and habitat preferences, life history, pathogenicity and population regulation in species of *Protocalliphora* Hough (Diptera:Calliphoridae). Dissertation. Utah State University, Logan, Utah.

198. Whitworth, T. L., and G. F. Bennett. 1992. Pathogenicity of larval *Protocalliphora* (Diptera:Calliphoridae) parasitizing nestling birds. Canadian Journal of Zoology 70:2184-2191.

199. Whitworth, T. L. 2002. Two new species of North American *Protocalliphora* hough (Diptera:calliphoridae) from bird nests. Proceedings of the Entomological Society of Washington 104:801-811.

200. Wikel, S. K., and D. Bergman. 1997. Tick-host immunology: significant advances and challenging opportunities. Parasitology Today 13:383-389.

201. Wikel, S. K., and F. J. Alarcon-Chaidez. 2001. Progress toward molecular characterization of ectoparasite modulation of host immunity. Veterinary Parasitology 101:275-287.

202. Wilson, C. M., and F. M. A. McNabb. 1997. Maternal thyroid hormones in Japanese quail eggs and their influence on embryonic development. General and Comparative Endocrinology 107:153-165.

203. Wilson, F. E., and B. D. Reinert. 1993. The thyroid and photoperiodic control of seasonal reproduction in American tree sparrows (*Spizella arborea*). Journal of Comparative Physiology Part B 163:563-573.

204. Wilson, F. E., and B. D. Reinert. 2000. Thyroid hormone acts centrally to program photostimulated male american tree sparrows (*Spizella arborea*) for vernal and autumnal components of seasonality. Journal of Neuroendocrinology 12:87-95.

205. Winkler, D. W., and F. R. Adler. 1996. Dynamic state variable models for parental care .1. A submodel for the growth of the chicks of passerine birds. Journal of Avian Biology 27:343-353.

206. Wong, G. K., and M. J. Cavey. 1993. Development of the liver in the chicken embryo.II. Erythropoietic and granulopoietic cells. The Anatomical Record 235:131-143.

207. Woodhead, A. D., R. B. Setlow, and V. Pond. 1982. Effects of polycyclic aromatic hydrocarbons on the proliferation of ectopic thyroid tissue in *Poecilia Formosa*, the Amazon Molly. Journal of Fish Biology 20:455-463.

208. York, R. G., W. R. Brown, M. F. Girard, and J. S. Dollarhide. 2001. Two-generation reproduction study of ammonium perchlorate in drinking water in rats evaluates thyroid toxicity. International Journal of Toxicology 20:183-197.

APPENDIX 1.

Bird species recorded at the Hummock Wetlands area (Golder Associates 1997)

Bird Type	**Common name**	**Latin name**
Shore birds	Common snipe	*Gallinago gallinago*
	Greater yellowlegs	*Aythya collaris*
	Kildeer	*Charadrius vociferus*
	Sora	*Porzana carolina*
	Spotted sandpiper	*Actitis macularia*
	Wilson’s phalarope	*Phalaropus tricolor*
Ducks	American wigeon	*Anas americana*
	Bufflehead	*Bucephalus albeola*
	Common Goldeneye	*Bucephala clangula*
	Green-winged teal	*Anas crecca*
	Lesser scaup	*Aythya affinis*
	Mallard	*Anas platyrhynchos*
	Northern pintail	*Anas acuta*
	Northern shoveler	*Anas clypeata*
Other aquatic spp.	American coot	*Fulica americana*
	Black tern	*Chlidonias niger*
	Horned grebe	*Podiceps auritus*
Raptors	American kestrel	*Falco sparverius*
	Long-heared owl	*Asio otus*
Sparrows	Chipping sparrow	*Spizella passerina*
	Clay-colored sparrow	*Spizella pallida*
	Le Conte’s sparrow	*Ammodramus leconteii*
	Savannah sparrow	*Passerculus sandwichensis*
	Song sparrow	*Melospiza melodia*
	White-throated sparrow	*Zonotrichia albicollis*
Other Passerines	Alder flycatcher	*Empidonax alnorum*
	American crow	*Corvus brachyrhynchos*
	American robin	*Turdus migratorius*
	Barn swallow	*Hirundo rustica*
	Brewer’s blackbird	*Euphagus cyanocephalus*

Bird Type	**Common name**	**Latin name**
	Brown-headed cowbird	*Molothrus ater*
	Cedar waxwing	*Bombycilla cedrorum*
	Common raven	*Corvus corax*
	Least flycatcher	*Empidonax minimus*
	Pine siskin	*Carduelis pinus*
	Red-breasted nuthatch	*Sitta canadensis*
	Red-winged blackbird	*Agelaius phoeniceus*
	Ruby-crowned kinglet	*Regulus calendula*
	Swainson’s thrush	*Catharus ustulatus*
	Tennessee warbler	*Vermivora peregrina*
	Tree swallow	*Tachycineta bicolor*
	Yellow warbler	*Dendroica petechia*
Woodpeckers	Northern flicker	*Colaptes auratus*