Impacts of Exposure to Polycyclic Aromatic Compounds (PACs) and their Alkylated Congeners in North American River Otter (*Lontra canadensis*)

By

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Abstract

Polycyclic aromatic compounds (PACs) are a group of chemicals encompassing thousands of different aromatic, alkyl aromatic and heterocyclic hydrocarbons (i.e.-containing N, S, or O- atoms); 16 of which have been designated as priority pollutants due to their toxicity and prevalence. Several studies have highlighted increases in the concentrations of C1-C4 alkylated PACs and heterocyclic aromatic hydrocarbons such as dibenzothiophenes in the atmosphere, water, soil and sediments, plants, wildlife and fish in the Athabasca Oil Sands Region (AOSR). Although there has been considerable research attention related to the toxic, carcinogenic and mutagenic properties of PACs, there is an increasing awareness that these chemicals may also have profound endocrine disrupting properties in wildlife. North American river otter (*Lontra canadensis*) are good indicators of ecosystem health due to their ecology and sensitivity to environmental pollutants. In this thesis, we first demonstrated the utility of adopting paleotoxicological frameworks in defining environmental baseline levels of PACs and likely biological effects from exposure to these complex environmental mixtures. These methods allowed us to reconstruct historical PAC deposition patterns to impacted areas while simultaneously determining likely biological effects such as endocrine disruption. Next, we showed how PACs exhibited trophic dilution in a Boreal food chain dominated by river otters. Snails, prey and predator fish, as well as river otters were collected from four main study areas in the AOSR in northeastern Alberta, Canada. Bioaccumulation factors such as biota-sediment accumulation factors (BSAF) and trophic magnification factors (TMF) were used to evaluate the partitioning behavior of PACs in the

environment and subsequent risks to biota. Our results revealed localized enrichment of certain PACs and subsequent metabolism in higher order vertebrates. Finally, we successfully combined ecotoxicological and physiological analyses paired with population genetic estimates to investigate endocrine disruption and population-level responses to exposure to PACs. River otters are known for their habitual use of latrine sites. Latrine sites represent a unique opportunity for biomonitoring programs to study river otters using indirect sampling methods. In this thesis, PACs were characterized and evaluated in sediment, lower and higher trophic biota with demonstrated impacts on endocrine processes and river otter population health. Effects-based assessments such as the ones presented in this thesis are more powerful for environmental monitoring programs than stressor-based assessment methods (such as describing presence/absence or levels of contaminants) as they provide greater biological context to monitoring data. In turn, these are helpful in selecting triggers for environmental effects monitoring or adaptive management programs.

Keywords: river otter; polycyclic aromatic compound; paleotoxicity; bioaccumulation; biomagnification; endocrine disruption

Dedication

I would like to dedicate this thesis to the many Cree, Dene, and Metis elders and community members who have opened their hearts, opened their homes, and shared their traditional knowledge that shaped the work in this thesis. Your infinite wisdom will forever be a source of inspiration.

Honor the Treaties

Only when

The last tree

Has died

And

The last river

Been poisoned

And the last fish

Been caught

Will we realise

We cannot

Eat money.

Cree Indian Proverb

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List of Acronyms

AHET	Alkylated Heterocyclic Aromatic Compound
AHMW	Alkylated Higher Molecular Weight
AhR	Aryl Hydrocarbon Receptor
ALMW	Alkylated Lower Molecular Weight
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
AOSR	Athabasca Oil Sands Region
APAC	Alkylated Polycyclic Aromatic Compound
ASE	Accelerated Solvent Extraction
ATH	Lake Athabasca
BACI	Before-after-control-impact
BaP	Benzo(a)pyrene
BL	Burnt Lakes
BSAF	Biota Sediment Accumulation Factor
CALUX	Chemical Activated Luciferase gene eXpression
CCME	Canadian Council of Ministers on the Environment
CFCS	Constant Flux Constant Sedimentation
CMR	Capture-mark-recapture
CSS	Cyclic Steam Stimulation
d.w.	Dry weight
DCM	Dichloromethane
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DRE	Dioxin-Response Element
EDC	Endocrine Disrupting Compound
EEM	Environmental Effects Monitoring program
EI	Electron Ionization
ERα	Estrogen Receptor Alpha
FCS	Fetal Calf Serum
FTS	Flow-to-surface
GC-MS	Gas Chromatography-Mass Spectrometry
GIS	Geographic Information System

GPC	Gel permeation chromatography
l.w.	Lipid weight
LLB	Lac La Biche
MOS	Mineable Oil Sands
mtDNA	Mitochondrial DNA
PAC	Polycyclic Aromatic Compound
PAD	Peace Athabasca Delta
PAH	Polycyclic Aromatic Hydrocarbon
PHET	Parent Heterocyclic Aromatic Compound
PHMW	Parent Higher Molecular Weight
PLMW	Parent Lower Molecular Weight
POP	Persistent Organic Pollutant
SAGD	Steam-Assisted Gravity Drainage
SAL	Saline Lake
SI	Stable Isotope
SMA	Surface Mineable Area
TEF	Toxicity Equivalent Factor
TEQ	Toxicity Equivalent Quotient
TL	Trophic Level
TMF	Trophic Magnification Factor
TOC	Total Organic Carbon
UHP	Ultra-high purity
USEPA	United States Environmental Protection Agency
w.w.	Wet weight

Chapter 1.

Introduction

Polycyclic aromatic compounds (PACs) are ubiquitous environmental contaminants released to the environment from a variety of sources, including but not limited to industrial air and water emissions, the combustion of organic matter (such as from forest fires), and fossil fuel combustion (such as vehicle exhaust). The production, transportation and environmental spills of oil and diluted bitumen rich in PACs are another important source of these environmental contaminants. For example, a spill of 1,000,000 L (1000 m^3) of crude oil containing 1% PAC by weight (mid-range; [1]), would introduce 10,000 kg (10 T) of PACs to a receiving environment. The Air Pollutant Emission Inventory estimated that 110 000 kgs of only four PACs (benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, and indeno[1,2,3-cd]pyrene) were emitted in Canada from anthropogenic sources in 2017 [2], which would represent only a small fraction of total PAC emissions. Despite an overall reduction in PAC emissions in Canada as a whole [2], in some sectors, PAC emissions are increasing, such as in the Oil Sand region [3]. Further, shipping volume of crude oil has been increasing in Canada, with volumes of exports increasing from 291.9 to 570.6 x 103m^3/day, from 2009 to 2018 [4], increasing the risk of environmental spills. Hence, there is still an urgent need for effective risk assessments to understand and limit the potential effects of PAC emissions on Canadian environments.

One of the main objective of chemical risk assessments is to define the concentrations in different environmental media that have the potential to cause harm to plant and animal species that occupy different ecosystems. These assessments are often challenged by the diversity of PAC compounds form different sources, the diversity of receiving environments and biota, and the diversity of mechanisms of toxicity. These assessments are made even more difficult by the fact that PACs never occur as single compounds in the environment, but as part of complex mixtures with thousands of other PACs and various other environmental contaminants interacting with each other.

Although some contaminants can degrade over time, others can be very stable and persistent in the environment. These toxic contaminants are of particular concern to top trophic predators that are susceptible to exposure to high levels through bioaccumulation and biomagnification processes. As a result, these compounds are of importance to risk assessments due to their longevity and greater probability of occurring as complex mixtures in the environment [5]. The exposure to complex mixtures of environmental pollutants adds a level of complexity when modelling risk of adverse health effects in wildlife.

When chemicals are found in the environment as complex mixtures, the mode of effect on exposed biota can fall under any of three main models of mixture toxicity. Under a non-interactive model, concentration addition and independent action result in a toxic effect equal to the sum of the contribution from each compound [5]. In interactive models, synergism/potentiation or antagonism processes results in mixture toxicity that

differs from the additive (non-interactive) assumptions. In these cases, with mixture components influencing each other, overall toxicity is either greater or weaker than expected [5]. Environmental risk assessments are complicated, and need to consider all possible modes of effect, for multiple compounds, often found as complex mixtures in the same environmental compartment.

Over the past decades, several strategies have evolved to overcome problems related to complex mixture assessments in screening processes and environmental risk assessments. Tools capable of providing rapid and reliable substance detection, identification and subsequent ecotoxicological evaluations in higher trophic biota are needed to inform robust risk assessment frameworks in Canada. As such, this thesis will explore not only organismal and population-level effects from exposure to environmental mixtures of PACs, but will also provide the necessary tools to historically evaluate the nature of these biological changes using paleolimnological methods.

1.1. Background

1.1.1. Polycyclic Aromatic Compounds (PACs)

Polycyclic aromatic compounds (PACs) are a group of chemicals encompassing thousands of different aromatic, alkyl aromatic and heterocyclic hydrocarbons (i.e.-containing N, S, or O- atoms); 16 of which have been designated as priority pollutants by the United States Environmental Protection Agency (USEPA), the US Agency for Toxic Substances and Disease Registry (ATSDR), and the European Food Safety Authority (EFSA) due to their toxicity [6–8]. PACs are also listed under Schedule 1 of the Canadian

Environmental Protection Act because of their high toxicity and the need to regulate their release to the environment. As a result of their hydrophobicity and lipophilicity, their capacity for long-range transport, their toxicity to aquatic organisms, their bioaccumulation potential and their strong interactions with sedimentary organic carbon, PACs have also been included in the Convention for Long-Range Transboundary Air Pollution's (CLRTAP's) Persistent Organic Pollutants (POP) protocol [9].

PACs are released into the environment through natural and anthropogenic origins from both petrogenic and pyrogenic sources. PACs are ubiquitous in the environment and are found primarily as complex mixtures, from environments as disparate as sediment in industrialized harbours to sea ice in the Arctic. Pyrogenic PACs are formed as a result of the rapid, incomplete and high-temperature combustion of organic matter (ex: wildfires and residential wood burning, vehicle exhaust, and municipal and industrial waste incineration). These pyrogenic PACs are thought to form from the 'cracking' of organic matter to lower molecular weight radicals, followed by the rapid reassembly into non-alkylated structures during pyrolysis [10]. This pyrolytic cracking also often produces soot carbon [10] that establishes strong interactions with other PACs, resulting in changes in PAC partitioning and bioavailability in the aquatic environment. Petrogenic PACs are formed by diagenetic processes over geological time scales, at relatively low temperatures and under high-pressure conditions (often leading to the formation of fossil fuels) [11]. These compounds are often alkylated, reflecting the ancient plant material (and biogenic precursors such as plant terpenes) from which the compounds were formed [10]. Being natural constituents of crude oil and related products, petrogenic PACs enter

the environment through their extensive transportation (by air, rail, pipeline, barge, or marine vessel), storage, and the use of crude oil and crude oil products [12]. Some of their major sources in the natural environment include oceanic and freshwater oil spills, natural seepages, underground and above ground storage tank leaks, pipelines, and the cumulative impacts of vast numbers of small releases of gasoline, motor oil, and related substances associated with transportation (ex: spills at gas stations, ship bilge pumping, etc) [12].

The ecosystems at highest risk for continuous PAC exposure are those close to urban areas (harbours, rivers in industrialized cities), at locations with a relatively high risk of catastrophic releases, such as from oil spills or tailings dam breaches, or at locations with high intensity industry, such as the oil and gas resource sector. For example, one study found that ∑15 PACs in soils were up to 60X higher in soil samples collected from urban vs. rural sites [13]. Because most PACs are bound to particles, sediments and soils are often the final sinks for these compounds through deposition and sedimentation processes [14]. Importantly sediment cores can be used to determine the temporal accumulation of PACs in an ecosystem. For example, urban sediment cores from Washington (dated back 200 years) showed increasing PAC loadings in the industrial era (after 1930) [15].

Recent studies have highlighted a substantial increase in PACs (especially the more polar, alkyl aromatic hydrocarbons, and hetero-compounds) at lower trophic levels during the past 25 years [16]. Indeed, recent evidence from the Norwegian Arctic suggests that

PACs now dominate the ∑contaminant burdens in lower trophic biota [16]. PACs have been detected in mammalian and non-mammalian wildlife populations, with sources linked to combustion (ex. forest fires) and petrogenic inputs (ex. oil and gas production) [17–20]. However, there is increasing concern globally regarding the impact of conventional and unconventional oil and gas extraction processes (e.g. bitumen processing, fracking) on PAC deposition in the environment [21–23]. In Canada, the Athabasca River and the Peace–Athabasca Delta region receives high PAC emissions from industrial development in the oil sands upstream region. Recent reports of increasing PACs in the Peace-Athabasca Delta following intensification of oil sands industrial activity [24] have raised concerns on the impacts of these hydrocarbons on Canadian fish and wildlife [25–27] as PACs are known to be genotoxic, mutagenic, carcinogenic and adversely impact reproductive success [28].

1.1.2. Mechanisms of PAC Toxicity

Regardless of the source of exposure to PACs their final tissue distribution/accumulation is a result of the passive diffusion of the compounds across membranes. This is attributable to the non-polar nature of PACs and the lipophilic characteristic of these compounds and cellular lipid membranes. Interestingly, however, the tissue distribution of PACs appears to be compound-specific and does not show a clear correlation with the lipophilic properties of the compound. For example, identical waterborne exposures of the fish, *Rasbora daniconius*, to naphthalene (NAP) and anthracene (ANT) revealed that NAP was accumulated almost exclusively in the intestine,

whereas ANT was mostly accumulated in the liver and kidneys [29]. Based on these general differences in tissue distribution, it can be expected that the toxicity of PACs will vary based on the capacity of these tissues to metabolize and detoxify PACs.

In mammals, the toxicity of PACs, for the most part, depends on the bioactivation of the parent compounds (through phase I and phase II metabolisms) into genotoxic, carcinogenic, and reactive oxygenated metabolites (see reviews by [30]; [31]). These reactions are universally mediated by the mixed-function oxygenase (MFO) family of enzymes such as cytochrome P450 (CYP). As phase I enzymes, CYP is central in the hydroxylation of these PACs, thus allowing for further modifications by other phase I and II enzymes (ex: GSTs, UDPGT and STs) into more soluble and excretable metabolites. As such, the metabolism of many PACs, particularly benzopyrene congeners [32–35], produces reactive oxygenated intermediates (e.g., epoxides and dihydrodiols) often with enhanced toxicity compared to the parent compound.

The role of CYP in PAC metabolism has been studied extensively. PACs have the ability to alter CYP1 metabolic pathways upstream of CYP1 induction. This induction is mediated by aryl hydrocarbon receptors (AhR). AhR are transcription factors that are activated after binding with coplanar PACs, or PACs with specific physicochemical properties [36]. Specifically, PACs with high affinity to AhR bind to this protein and to two molecules of heat-shock protein 90 (HSP90) which acts as a molecular chaperone. This PAC-AhR-HSP90 complex is then translocated to the nucleus of the cell where it loses its HSP90 chaperones and binds to the AhR co-factor, the aryl hydrocarbon receptor nuclear translocator

(ARNT). This PAC-AhR-ANRT complex finally binds to a DNA recognition sequence upstream of the CYP genes, also known as the xenobiotic response element (XRE), increasing the transcription of the CYP genes and subsequent protein translation. Similarly, there are phase II enzymes (e.g. GSTs and UDPGTs) whose regulation is also mediated through the AhR pathway [37–39]. The affinity of PACs to AhR depends on their physicochemical properties. For example, a study that investigated the induction of CYP1A1 and CYP1A2 enzyme activity in human cell lines demonstrated that PAC molecules with aligned rings exhibited reduced affinity to AhR, and were thus less potent inducers of CYP1A compared to a PAC with clustered rings [40]. The bay-region of the PAC molecule is also considered as an important promoting factor of CYP1A induction since it confers higher affinity to the AhR active binding site [40]. For some PACs, such as lower molecular weight PACs that have little or no activity as AhR ligands (e.g. devoid of a bay-region), toxicity appears to be AhR-independent, thus very mechanistically distinct from higher molecular weight PAC compounds [41]. However, there are exceptions, such as fluoranthene [42], carbazole, and dibenzothiophene [43], where coplanar PACs have no, or almost no, affinity for the AhR, and can even act as CYP inhibitors.

1.1.3. PACs in the Oil Sands

Because of increasing anthropogenic activities and disturbances, petrogenic and pyrogenic PACs are being emitted at ever-growing levels, and their concentration in the broader environment has increased considerably over recent decades [44]. PACs are indeed found ubiquitously in both aquatic and terrestrial environments [45]. However, as

is the case with many environmental pollutants, PACs are never found alone in the environment, but as components of complex mixtures that contain hundreds if not thousands of different compounds. The hazard assessment of complex mixtures (including PACs) is not a simple task as many compounds in a mixture have drastically different chemical properties, modes of toxicity, and potential to interact (through synergistic and antagonistic interactions) *ex- and in-vivo*.

The importance of understanding the impacts of PACs cannot be understated, especially in a world that is increasingly reliant on energy produced from the burning of fossil fuels which is resulting in increased releases of these petrogenic PACs into the environment. As is the case for many other environmental pollutants, the effects and underlying mechanisms triggered by exposure to PACs have been mostly drawn from experimental research under laboratory conditions, for single compounds, where ecological relevance was often omitted (in terms of concentrations, model organisms, and most importantly, PAC mixtures). Also, despite the fact that there are many PAC compounds known to science, the bulk of the research effort has historically focused on a few representative substances (e.g. list of 16 USEPA priority PACs) and has largely ignored many of the other compounds which have been increasing in the environment as a result of anthropogenic activity, such as alkylated PACs. The oil sands deposits in Northern Alberta (Canada) offer a unique opportunity to study the impacts of complex PAC mixtures as the region is influenced by both natural and anthropogenic sources of PACs.

The western Canadian sedimentary basin contains about 2.3 trillion barrels of natural bitumen rich in PACs [46]. This is equivalent to roughly 43% of the known global oil reserve, 170 billion barrels of which are economically recoverable by the province of Alberta [46]. With annual bitumen production increasing, even in an environment of low oil prices, large volumes of OSPW and fine tailings (the silt/clay fraction of bitumen extraction) are stored in tailings ponds [47]. Oil sands mining operators are further required to reclaim their leases through strategies including “wet landscape” approaches that involve the creation of lakes lined with fine tailings that will be enriched in PACs and heterocyclic aromatic compounds that are then capped with layers of freshwater [47]. Concerns have been raised about the potential toxic effects from exposure to PACs in fish and aquatic wildlife in these reclaimed landscapes [47].

Canada’s oil sands deposits are located primarily in the Peace River, Cold Lake, and Athabasca regions of northern Alberta, Canada [48]. Of these, the Athabasca Oil Sands Region (AOSR) is the largest and has undergone the most intensive development. The AOSR is situated wholly within Canada’s boreal forest in the Mackenzie watershed, which is home to multiple Indigenous communities living in and around the region. Ecologically significant areas such as the Peace-Athabasca Delta (PAD; designated as a Ramsar Site under the RAMSAR Convention) and Wood Buffalo National Park (a UNESCO World Heritage Site) lie downstream of the AOSR [49]. Production in the Alberta oil sands has increased significantly, with recent forecasts estimating that the resource will contribute more than 1.7 trillion dollars to Canada’s economy over the next decade [50]. However, despite the economic benefits of the oil and gas industry, there remain significant

concerns regarding the impacts of increased oil production on the environment and human health [25,51–53]. In particular, the deposition of polycyclic aromatic compounds (PACs), including heterocyclic aromatic compounds containing N-, S- and O- atoms, to the abiotic environment has raised concerns regarding their impacts on surrounding wildlife and ecosystem health.

The oil sands are naturally occurring mixtures of crude bitumen (thick, heavy crude oil), sand, clay, ultrafine mineral solids and water. Bitumen contains polycyclic aromatic hydrocarbons (PAHs), alkylated PAHs and dibenzothiophenes; collectively known as petrogenic bitumen-derived polycyclic aromatic compounds (PACs; [54]). There are two sources of bitumen-derived PACs to the environment, those released naturally from oil sands deposits (i.e., due to erosion) and those from bitumen extraction and upgrading (i.e., oil sands activity). Pyrogenic PACs can also be released to the environment as a by-product of the rapid, high temperature and often incomplete combustion of organic matter [10,24,26,55].

1.1.4. Identification and Characterization of PACs in the Oil Sands Region

Understanding the depositional patterns (including deposition rates) to the abiotic environment in the oil sands is important in order to properly evaluate potential exposure to wildlife. Careful evaluations of the levels of bitumen-derived PACs in abiotic matrices allows us to further refine our exposure estimates to the biotic environment in order to obtain more robust risk estimates. A literature review was undertaken to synthesize information regarding PACs in the air (including atmospheric deposition modelling), water

(including snow) and sediment (including the soil) in the oil sands region. The search strategy included peer-reviewed literature published in the last 20 years, identified from searches of Pubmed, Scopus and Google scholar using the following combination of keywords: “oil sands”, “polycyclic aromatic hydrocarbon”, and “polycyclic aromatic compound”. Only the studies providing PAC levels obtained through GC-MS (or higher equivalent) using an internal standard method (when available) in abiotic matrices were retained for analysis and discussion. A total of 30 studies were identified for this analysis.

The use of diagnostic ratios has steadily increased over the last decade as a qualitative tool to assess PAC sources to the aquatic ecosystems [56]. While some limitations exist in their use and interpretation, when coupled to compound-specific isotopic analysis (CSIA), they can discriminate between pyrogenic and petrogenic PAC sources to the environment [56,57]. In the sediment record, most studies agree that recent post 1960 PAC inputs temporally shifted from primarily wood combustion sources (i.e., pyrogenic sources) to petrogenic inputs coinciding with oil sands development [22,24,55,56].

1.1.5. Bitumen-derived PACs in Air (including atmospheric deposition modelling)

Following some of the early studies by Kelly et al. (2009) on the effects of oil sands mining on the deposition of PACs to the Athabasca River Basin, the Federal Minister of the Environment convened the Federal oil sands Advisory Panel in 2010 to address pollution concerns from oil sands activities in Alberta [26,58]. As a result of some of the recommendations brought forward by the Panel, an environmental monitoring plan for the Lower Athabasca River and tributaries was developed. In 2011, Environment Canada

released the Integrated Oil Sands Environment Monitoring Plan, which focused on air quality, biodiversity and water quality in the AOSR. In 2012, the Joint Canada/Alberta Implementation Plan on oil sands Monitoring (JOSM) was released by Environment and Climate Change Canada and Alberta Environment and Parks and outlined a collaboration to phase-in monitoring activities.

One of the early initiatives under the Integrated Monitoring Plan was the establishment of a 17-site passive air sampling monitoring network to measure PACs in ambient air using a combination of PUF (polyurethane filters) passive air sampling devices. In those studies, PAC results were validated for both the gas-phase and particle-phase using calibrated values obtained through high volume samplers at a subset of sites [58]. The use of field blanks and duplicates were also always part of the experimental design.

Results generated by studies including Schuster et al. (2015), Hsu et al. (2015) and Jariyasopit et al. (2016) consistently noted an abundance of alkylated-PACs (APACs) and dibenzothiophenes (DBTs and alkylated congeners) over their parent compounds and an exponential decline in PAC concentration in air with distance from surface mineable areas [58–60]. These distance decay relationships were especially significant in APACs and DBTs, even during forest fire periods, suggesting an association to petrogenic sources [58]. This association is not too surprising, given their volatility and higher association with deposition particles [58]. Seasonal trends in PACs were noted in some studies [59], but not in others [58] while some studies suggested day/night trends in PAC composition and

distribution between gas-phase and particle-phase [61]. While levels ranged highest in PUF samplers near oil sands mining and upgrading facilities, the average PAC concentration in communities such as Fort McKay and Fort McMurray were within the range of typical rural and semirural areas across Canada [59]. Where PACs were most often linked to oil sands industrial sources, they were also associated with NO, NO2, PM2.5, and SO2, suggesting that combustion sources (such as industrial stacks, vehicles, residential heating, forest fires, etc.) are important contributors to ambient PACs in this region [59].

Numerous studies investigated the relative contribution of emission sources to ambient PACs in air in the oil sands region [62–66]. The most recent study identified five main sources as being the main contributors to PM2.5 (and as a result, total PACs in the air) [66]. These included biomass combustion > fugitive dust > upgrader stack emissions > petrogenic PACs > and transport aerosol [66]. Most studies agree that airborne petroleum coke dust emissions are the major contributor to total ambient PAC concentrations [62–64]. Petroleum coke (petcoke) dust emissions were often associated with increased heterocyclic aromatic compounds (including DBTs) [62], higher molecular weight PACs (including Benzo(a)pyrene, BaP) and certain heavy metals (like V) [63,64]. Despite indications that some studies may have overestimated air PACs [67], the significant contribution of petcoke dust emissions remains. As such, mitigating fugitive emissions from petcoke stockpiles may represent the greatest opportunity to reduce environmental loadings of PACs in the AOSR [67].

1.1.6. PACs in Water (including snow)

Of particular concern in the AOSR are inputs of PACs into aquatic ecosystems as a result of oil sands mining activities, pipeline releases and accidental spills. There were >9,262 environmental incidents in the AOSR between 1996 and 2012, [68] with 1,179 documented pipeline releases in the same study period [68]. Other accidents such as the October 31st 2013 tailings pond breach at the Obed Mountain Coal Mine (near Hinton, AB) where 670 million litres of coal slurry, rich in PACs, entered the environment continues to raise concerns. Compounded by some of the first, and early reports of seepage of oil sands process affected water (OSPW), which is also rich in PACs, from tailings ponds to the Athabasca River stresses the urgency to understand the levels, sources, fate and effect of bitumen-derived PACs in aquatic ecosystems of the AOSR [69,70].

An early report by Kelly et al. (2009) showed that oil sands development were a greater source of environmental contamination than previously reported. The authors reported in 2008 that within 50km of oil sands mining and upgrading, over a four-month period, 11,400T of airborne particulate, including 391kg of PACs was deposited in the snowpack [26]. Later studies demonstrated a distance decay relationship of PACs deposited to snowpack, with the highest levels reported in the geographic centre near Suncor and Syncrude upgraders [71,72]. Other research further provided evidence of PAC inputs from oil sands operations clustered around the Athabasca River, or along a north-south vs. west-east direction [71]. These aerial deposition models were further validated using HYbrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) modelling [71]. Similar

to other studies, PACs in snowpack were dominated by DBTs, phenanthrenes/anthracenes (PHE/ANT), fluranthenes/pyrenes (FLU/PYR), chrysenes (Chr), and fluorenes (Fl) [70–72]. The PACs detected were highly dominated by their alkylated congeners, especially near oil sands operations, and were attributed to particulates associated with mining activities [71,73]. Manzano et al. (2017) further determined that sites <30 km from mining operations showed a similar distribution of heterocyclic aromatic compounds (such as benzonaphthothiophenes; BNThs) to petcoke. A more concentrated spatial distribution pattern for these compounds suggested a more rapid atmospheric deposition process, implying a larger particle size, consistent with petcoke windborne fugitive particles [62].

Compositional differences in PACs and other organic compounds deposited to snowpack were detected for near-field sites, and far-field sites [71,74]. Results from these studies showed that site location influenced the PAC profile in the snowpack. Further diagnostic ratio analyses provided evidence that oil sands, and non-oil sands sources combined in the PAC deposition [71]. Later, Birks et al. (2017) showed that compositional differences in PACs differed based not only on location, but were also different depending on whether the sample was snow or freshwater. Their dataset included total dissolved and particulate PACs (including 34 parent and alkylated-PACs) generated across 105 snow, 272 river, and 3 lake samples [70]. Seasonal differences in PACs were observed depending on flow conditions and snowmelt [70]. However, because the snowpack PAC composition differed from river PAC profiles during peak periods, the authors suggested that snow may not be the major source of PACs to the Athabasca River and its tributaries

[70]. Instead, they suggested that snowmelt may indirectly contribute to increased PACs in the Athabasca River through other hydrological processes such as erosion of stream channels, remobilization of PAC containing sediments and increased catchment runoff, amongst others [70].

1.1.7. PACs in Sediment (including soil)

Aquatic sediment cores can provide information on the temporal trends of contaminant input to the environment [75]. Sediment cores are especially important when baseline long term monitoring data are missing [75]. Similar to air and water matrices, alkylated PACs were generally more abundant than their parental homologues in AOSR sediment [24,47]. The dominating nature of alkylated PACs over parent compounds is diagnostic of petrogenic inputs, typically associated with oil deposits as was demonstrated in previous studies in the AOSR [47,73]. PACs in sediments are often correlated to sedimentary organic carbon, and recent estimates suggest that over the last few decades, both sedimentary organic carbon, and PACs in sediment steadily increased after the development of the bitumen resource [22,24,46,55,56]. This increase has been noted in lake sediments up to 90 km from mining operations and has further been detected up to 200 km downstream in the Peace Athabasca Delta for certain diagnostic PAC compounds (such as phenanthrene) [24,56]. Increases in alkylated-PACs in the sediment record was usually followed by increases in DBTs and other heterocyclic aromatic compounds, typical of anthropogenic inputs of bitumen-derived PACs, such as those that are indicative of fugitive petcoke dust emissions [22,24,62].

Few studies have focused on PAC sources and levels outside the surface mineable area (SMA) of the AOSR, with the exception of a few key sites near Cold Lake, Alberta [76,77]. At locations outside the SMA, in situ extraction technologies are used to extract bitumen from greater depths. Despite a greatly reduced mining environmental footprint, these studies nevertheless determined distance decay relationships in PACs from individual extraction rigs [77]. Similar to PAC signatures in the SMA, an enrichment in alkylated PACs over their parent compounds and the shift from predominately pyrogenic signatures to petrogenic signatures provide further evidence that in situ extraction operations contribute to PAC burdens in aquatic ecosystems at the local level [76]. In their study, Korosi et al. (2016) documented probable flow-to-surface incidents (FTS; or the appearance of bitumen emulsions from deep subsurface recovery zones at the surface of freshwater bodies). This highlights the need to better understand the potential role of in situ extraction as a source of PACs to landscapes and surface waters [76].

1.1.8. PACs as Endocrine Disrupting Chemicals (EDCs)

Several studies have highlighted increases in the concentrations of C1-C4 alkylated PAHs and heterocyclic aromatic hydrocarbons such as dibenzothiophenes (prominent components of AOSR bitumen [54]) in the atmosphere, water, soil and sediments, plants, wildlife and fish in the AOSR as a result of proximity to oil sands industrial activity [17,18,24,26,55,78–81]. Although there has been considerable research attention related to the toxic, carcinogenic and mutagenic properties of PACs, there is an increasing awareness that these chemicals may also have profound endocrine disrupting properties

in PAC-exposed wildlife [82–84]. This is especially true of the polar polycyclic aromatic hydrocarbons (such as heterocyclic aromatic hydrocarbons containing N-, S- or O- atoms) and their metabolites that are known as endocrine disruptors through 7-ethoxyresorufin O-deethylase (EROD) induction and estrogenic activities [33,85]. Increasing evidence suggests that crude oil-related contaminants such as PACs and other heterocyclic aromatics may lead to adverse health effects in freshwater ecosystems as a result of altered endocrine signaling, such as those related to thyroid hormone system, adipogenesis, and reproduction [86].

Environmental PACs can be considered as EDCs due to their ability to interfere with the homeostasis of endogenous hormones, especially those tied to female reproductive function [87,88]. Impaired placental functions such as reduced endocrine and metabolic activity of the placenta were noted in women living in environments with elevated levels of aromatic hydrocarbons [89]. In another study, benz(a)anthracene, benzo(a)pyrene and fluoranthene were shown to behave as estrogen-like compounds in immature Wistar rats [90]. It was later demonstrated that different types of crude oils exhibit antiestrogenic and antiandrogenic activity in in vitro models [86,91]. Using a more targeted approach (in terms of the oil-related compounds tested), 5 major PACs and their alkylated congeners commonly detected in crude oil were tested in MVLN-luc and H295R cell lines to determine their estrogen receptor (ER)-mediated potency and capacity to disrupt endocrine function [83]. The authors demonstrated that for major PACs and their alkylated congeners, disruption of steroidogenic pathways appeared to be more significant than ER-mediated responses to PAC exposure [83].

Many PACs are aryl hydrocarbon receptor (AhR) agonists, like dioxin and dioxin-like compounds, which explains some of the most toxic effects from PAC exposure [83,92]. PACs can have both anti-estrogenic effects via induction of AhR dependent cytochrome p450 enzymes (incl. CYP1B1, CYP1A2, CYP3A4, and CYP1A1) that metabolize estradiol, and estrogenic effects through metabolism of the PACs to estrogenic metabolites, induction of aromatase (estrogen biosynthetic enzyme) and direct interactions with the estrogen receptor [93–98]. Furthermore, PAC metabolites such as hydroxylated PACs not only interact with estrogen receptors directly [99], they can also interact with other dioxin-like compounds in a mixture altering estrogen metabolism via crosstalk between the AhR and sulfotransferase drug metabolizing enzymes such as SULTE1 [96]. Ultimately, steroidogenic pathways are affected, and hormone homeostasis is impaired [96]. Perturbations in estrogen homeostasis and signaling can have profound effects on individual reproduction with subsequent population-level impacts.

In the oil sands, fish have often been used to study the impacts of exposure to fossil fuel-contaminated sediments [100]. In early wild-caught fish studies in the AOSR, endpoints such as gonadosomatic indices (GSI), fecundity, and in vitro gonadal steroid production were assessed [100]. Lower levels of steroid production were detected during *in vitro* gonadal incubation experiments at sites along the Steepbank River in the surface mineable area of the oil sands deposit. Hepatic EROD activity, used as an indicator of exposure to oil sands related contaminants such as PACs was elevated at sites closer to oil sands industrial activity compared to a reference site. No consistent alterations in gonadal development were detected in fish collected from sites in the AOSR [100].

Tetreault et al. (2003) provided some of the first baseline information on the health of fish populations in the AOSR. Under controlled laboratory conditions, rainbow trout hepatocytes exposed to extracts of oil sands process-affected water (OSPW; rich in PACs) revealed that CYP3A4 gene expression changes were more pronounced in fish exposed to OSPW vs Athabasca River water [101]. CYP3A4 expression was highly correlated to CYP1A1 and VTG (vitellogenin; a key yolk protein) expression, contributing to the endocrine disruption activity of these mixtures [101].

PAC-related endocrine disruption studies in the oil sands in higher-order vertebrates (such as birds and mammals) are scarce. In the few endocrine disruption-focused studies, Cruz-Martinez et al. (2015) demonstrated that nestling tree swallows (*Tachycineta bicolor*) had higher EROD induction at sites closer to industrial activity in the oil sands, similar to wild fish observations. Lower relative hepatic mass, a smaller T cell response to the phytohemagglutinin skin test, and smaller bursae of Fabricius were also noted [102]. These endocrine-disrupting changes from exposure to PACs were identified as one of many stressors impairing reproductive and developmental changes in tree swallows [103].

1.2. Biomonitoring

1.2.1. River Otters as Sentinel Species of Ecosystem Health

The Athabasca River and the Peace–Athabasca Delta may be impacted by industrial development in the upstream region of the oil sands. Recent reports of aquatic contamination have raised concerns on the impacts of PACs on wildlife [25,26]. The North

American River Otter (*Lontra canadensis*) is found throughout North America in both freshwater and marine environments. *L. canadensis* is listed in Appendix II of the Convention on International Trade or Endangered Species (CITES) due to their susceptibility to the impacts of trade, trapping, and other factors such as environmental contaminants and parasitic diseases [104].

Because river otter feeds predominately on fish at higher trophic levels and a variety of opportunistic prey, they are considered as keystone species in watersheds of the Boreal forest. They are also considered as a good bioindicator species (i.e., a sentinel species) of aquatic ecosystem health due to their trophic position and their relative sensitivity to environmental stressors such as environmental contaminants [105,106]. Intensive trapping of river otters in the nineteenth and early twentieth century as well as habitat destruction through industrial development (e.g., mining, oil and gas development, pulp and paper), and subsequent water pollution led to the decline and local extirpations of some populations in regions of North America [105,107,108].

As an apex mammalian predator, they may be susceptible to negative health consequences resulting from the biomagnification of persistent environmental pollutants through their diets [109–111]. A good indicator of environmental contaminants must mirror the contaminant levels in their environment and must be relatively cost-efficient to sample. Because river otters are relatively abundant in Alberta and North America, they fulfil both of these criteria. Further, they are economically and socio-culturally important furbearers for Indigenous Nations that rely on healthy ecosystems for subsistence [105].

1.3. Rationale and Objectives

Various studies have demonstrated the acute toxicity and reproductive toxicity of PACs to laboratory mammals [112,113]. Experimental studies in wild species have also demonstrated the potential toxic effects of PACs on other endpoints, some of which may be particularly important for free-living mammals, such as the suppression of immune function, altered food consumption and the presence of DNA adducts and associated increased carcinogenicity [114–117]. However, there are few published ecological studies investigating the chronic toxicity of PACs (including their alkylated congeners and heterocyclic aromatics) on wild mammal health and populations, none in apex predators in the AOSR.

There is evidence of increasing loadings of PACs via aerial deposition to aquatic environments in the AOSR. This is evident from measurements of snow, and sediment cores of lakes up to 90km away from mining operations. Increased loadings of PACs in these snow and sediment samples correlate to increases in mining activities since the 1960s. Yet little is known on the biological impacts (including endocrine-disrupting effects) of exposure to PACs, and most importantly, their alkylated congeners and heterocyclic aromatic compounds in higher trophic vertebrates. As such, this thesis will provide some critical information on the accumulation and biological impacts of chronic exposures to PACs in free-ranging river otters, a key species in the boreal ecosystem. Specifically, I will address:

Aim 1- Paleotoxicological Evaluation of Polycyclic Aromatic Compounds in Sediment Cores as Endocrine Disrupting Chemicals

The objectives of this section are 1- to determine a chronology of PACs in dated sediments from four freshwater lakes in the AOSR and control region, and 2- to determine the time-constrained endocrine responses resulting from exposure to these complex PAC mixtures. I will test the prediction that there will be temporal changes in the endocrine responses to sediment cores that correlate with increased industrial activity in the AOSR. To my knowledge, this study is the first to employ a paleotoxicological approach to determine the potential for toxicological effects from exposure to complex PAC mixtures in oil sands impacted areas.

Aim 2 - Bioaccumulation of Polycyclic Aromatic Compounds (PACs) in a Boreal food chain dominated by North American River Otter (*Lontra canadensis*)

The objectives of this section are 1- to determine the biota-sediment accumulation factors (BSAF) of a suite of PACs in snails from sediment, and 2- to calculate bioaccumulation factors (BAF) such as trophic magnification factors (TMF) to determine if these compounds undergo biomagnification in snails, prey and predator fish, and river otters as a representative apex predator. I will test the prediction that larger and heavier PAC compounds (with increasing alkylation) will bioaccumulate more than their parent compounds. To my knowledge, this study is the first to generate whole trophic level information on the levels of a suite of PACs, contrasting BSAF and BAF in a Boreal forest food chain impacted by complex PAC mixtures in the AOSR.

Aim 3 - River Otter population dynamics and hormonal responses in a landscape dominated by oil and gas anthropogenic disturbances

The objectives of this section are 1- to determine the dose-response relationships of exposure to environmental PAC mixtures on the levels of fecal hormone metabolites in river otter; 2- to monitor river otter population dynamics at high- and low-impact traplines using molecular genetic techniques; and 3- to determine the impacts of increased PAC burdens on river otter population dynamics. I will test the prediction that animals with higher levels of hepatic PACs will have different fecal hormone metabolite levels and that relationships between key PACs and fecal hormonal responses will mirror laboratory-derived results. Finally, I hypothesize that river otters living in areas with higher levels of environmental PACs will exhibit poorer population dynamics than otters living at more pristine locations. To my knowledge, this study is the first to link PAC exposure with population-level responses in free-ranging terrestrial biota in the AOSR. This study will also be the first to document dose-response relationships on the hormonal responses of wild mammals exposed to environmental PAC mixtures.

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Chapter 2.

Paleotoxicological Evaluation of Polycyclic Aromatic Compounds in Sediment Cores as Endocrine Disrupting Chemicals

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2.1. Abstract

Despite the economic benefits of the oil and gas industry in Northern Alberta, there remain significant concerns regarding the impacts of increased oil production on the environment and human health. Concerns regarding the impacts of contaminants arising from oil sands activity on surrounding wildlife and ecosystem health are consistently being raised. Several studies have highlighted increases in the concentrations of polycyclic aromatic compounds (PACs) in the atmosphere, water, soil and sediments, plants, wildlife and fish in the Athabasca Oil Sands Region (AOSR) as a result of proximity to oil sands industrial activity. PACs are of considerable concern for human and wildlife health due to their significant toxic, carcinogenic, mutagenic, and endocrine disrupting properties. Aquatic sediment cores can provide information on the temporal trends of contaminant input to the environment. Sediment cores are especially important when baseline monitoring data is missing. There has been little work to understand the biological impact of contaminants in these cores as a surrogate for the toxicity to freshwater systems. Using a paleotoxicological approach, our study combines analytical chemistry and a mammalian cell-based bioassay in dated lake sediment cores to assess paleotoxicity in freshwater systems affected by PACs emitted in the oil and gas sector and/or through natural forest fire and biomass combustion. Four sediment cores were collected from natural water bodies in the AOSR. Sediment intervals were radiometrically dated and subsequently analysed for PACs. PAC extracts from select dated intervals were used in cell-based bioassays to evaluate their endocrine disrupting properties. We demonstrated that there is spatial and temporal variability in the PAC composition of sediment cores around the

AOSR with some of the highest levels of PACs and biological activity being detected near oil sands industrial activity. Our study is the first to link chemical analysis of sediment cores with endocrine function showing the feasibility of extending the usefulness of sediment cores in monitoring programs. While we observed no spatial or temporal differences in ERα mediated signaling, this approach demonstrates the powerful potential of coupling biological effects assessments to historical reconstructions of contaminant inputs to the natural environment.

2.2. Introduction

Canada's oil sands deposits are located primarily in the Peace River, Cold Lake, and Athabasca regions of northern Alberta [1]. Of these, the Athabasca oil sands region (AOSR) is the largest and has undergone the most intensive development. The AOSR is situated entirely within Canada's boreal forest in the Mackenzie watershed, which is home to multiple Indigenous communities living in and around the region. Downstream of the AOSR lie ecologically significant areas including the Peace-Athabasca Delta (PAD; designated as a Ramsar Site under the RAMSAR Convention) and Wood Buffalo National Park (a UNESCO World Heritage Site) [2]. Production in the Alberta oil sands has increased at a staggering pace, and recent economic forecasts estimate that the resource will contribute more than 1.7 trillion dollars to Canada's economy over the next decade [3]. However, despite the economic benefits of the oil and gas industry, there remain significant concerns regarding the impacts of increased oil production on the environment and human health [4–7]. In particular, the Mikisew Cree First Nation in Fort

Chipewyan, Alberta, has repeatedly raised concerns regarding the impacts of contaminants arising from oil sands activity on surrounding wildlife and ecosystem health.

Indigenous communities downstream from oil operations in the PAD are concerned about the quality of the water due to increased anthropogenic inputs of contaminants from the oil and gas sector [7]. The concern is not only due to knowledge of accidental spills [8] but is also a result of first hand observations by Indigenous Knowledge holders of declines in the quality of the Athabasca River and its tributaries. Elders and land users have observed negative trends for Indigenous Knowledge Indicators such as more algae, foamy scum, dirtier water, scum on rim of the tea pots and boats, as well as a stronger smell to the water [7,9,10]. A rise in fish deformities [6,11] and reductions in numbers of fish and terrestrial wildlife, most notably those living in or near water courses (ex., muskrats [12]) raises fears in the community that the Athabasca River and its tributaries will no longer support the aquatic life so important to traditional Indigenous lifestyles [9,10] These concerns are supported by experimental studies which have shown that meltwater from snow and contact with sediments collected from sites close to mining and upgrading operations negatively impact survival and increase the number of deformities in fish species which are native to the Mackenzie River watershed [13–16].

The oil sands are naturally occurring deposits crude bitumen comprised of a mixture of thick, heavy crude oil), sand, clay, ultrafine mineral solids and water. Bitumen contains polycyclic aromatic hydrocarbons (PAHs), alkylated PAHs and dibenzothiophenes; collectively, these are known as polycyclic aromatic compounds (PACs). PACs are released

naturally from oil sands deposits (i.e., due to erosion of the bitumen deposits) and anthropogenically from bitumen extraction and upgrading (i.e., oil sands activity) as a by-product of the rapid, high temperature and often incomplete combustion of organic matter [17–20]. These are commonly referred to as petrogenic and pyrogenic PACs respectively. Several studies have highlighted increases in the concentrations of C1-C4 alkylated PAHs and dibenzothiophenes (prominent components of AOSR bitumen [21]) in the atmosphere, water, soil and sediments, plants, wildlife and fish in the AOSR as a result of proximity to oil sands industrial activity [14,18–20,22–26]. PACs are of considerable concern for human and wildlife health due to their significant toxic, carcinogenic, mutagenic, and endocrine disrupting properties [27–29].

Many PACs are aryl hydrocarbon receptor (AhR) agonists, like dioxin and dioxin-like compounds, which explains some of the most toxic effects from PAC exposure [29,30]. PACs can have both anti-estrogenic effects via induction of AhR dependent cytochrome p450 enzymes (incl. CYP1B1, CYP1A2, CYP3A4, and CYP1A1) that metabolize estradiol, and estrogenic effects through metabolism of the PACs to estrogenic metabolites, induction of aromatase (main estrogen biosynthetic enzyme) and direct interactions with the estrogen receptor [31–36]. Furthermore, PAC metabolites, such as hydroxylated PACs not only interact with estrogen receptors directly [37], they can also interact with other dioxin-like compounds in a mixture, altering estrogen metabolism via crosstalk between the AhR and sulfotransferase drug metabolising enzymes such as SULTE1 [35]. Ultimately, steroidogenic pathways are affected and hormone homeostasis is impaired [35]. Perturbations in estrogen homeostasis and signalling can have profound effects on

reproduction at an individual and population level. For-example, a 7-year whole-lake experiment at the Experimental Lakes Area (ELA; Ontario, Canada) revealed that chronic exposure to low concentrations (5–6 $ng \cdot L^{-1}$) of a potent synthetic estrogen (17α-ethynylestradiol) led to the feminization of male fathead minnows (*Pimephales promelas*) through the production of vitellogenin mRNA and protein. These changes led to impacts on gonadal development (including altered oogenesis in females) culminating in the near extinction of this fish species from the lake [38].

The importance of understanding the biological impacts of PACs is important as society becomes increasingly reliant on energy produced from the burning of fossil fuels. As is the case for many other environmental pollutants, the effects and underlying mechanisms triggered by exposure to complex PAC mixtures have been mostly drawn from experimental research under laboratory conditions, for single compounds, where ecological relevance was often omitted in terms of concentrations, model organisms, and most importantly, PAC mixtures. In spite of the many PAC compounds known to science, research also tends to focus on a few representative substances (e.g. USEPA priority PAHs), and not necessarily compounds most common in particular natural setting. PAC mixtures detected in the natural environment are not only complex in composition, but also in temporal variation (e.g. air/water can change hourly, daily, seasonally).

Aquatic sediment cores can provide information on the temporal trends of contaminant input to the environment [39]. Sediment cores are especially important when baseline long term monitoring data is missing [39]. However, there has been little

work to understand the biological impact of the contaminants in these cores as a surrogate for the toxicity to the freshwater systems. Using a paleotoxicological approach [39], our study combines analytical chemistry and a mammalian cell-based bioassay in dated lake sediment cores to assess paleotoxicity in freshwater systems affected by PACs emitted in the oil and gas sector and/or through natural forest fire and biomass combustion. Specifically, our objectives are 1- to determine a chronology of PACs in dated sediments from 4 freshwater lakes; and 2- to determine the time-constrained toxic response from exposure to these complex PAC mixtures. To our knowledge, our study is the first to employ a paleotoxicological approach to determine the potential for toxicological effects from exposure to complex PAC mixtures in oil sands impacted areas. With this approach, we hope to assess potential inherent risks to endocrine function which in turn might inform potential risks to reproductive fitness from exposure to natural and anthropogenic PAC mixtures.

2.3. Materials and Methods

2.3.1. Study Area

Geologically, all the sampling sites are located in or near the McMurray Formation, a stratigraphic unit of the early Cretaceous period of the Western Canada Sedimentary Basin [40]. It was during the late Jurassic to mid-Cretaceous periods that the province sank, allowing the Arctic Ocean to migrate south and cross into northern Alberta until it eventually connected with the Gulf of Mexico 85 million years ago [40,41]. The river and oceanic processes, along with the deposition of fine clays and sediments, eventually

contributed to the formation of the bitumen-rich McMurray Formation. As a result, the region is home to the largest oil sands deposit in Alberta, covering an area of about 46,000 km^2 [41]. The four freshwater lakes selected in our study ranged from small (<3km^2) to very large (>100km^2; Table 2-1). All were sites identified by traditional land users as being important for the practice of cultural and recreational activities (e.g. hunting, fishing and trapping). Three sites were located in the Athabasca Oil Sands Region (AOSR) in northeastern Alberta, Canada and one was at Lake Athabasca, a downstream site just outside the AOSR deposit. Inside the AOSR, one lake (Lac La Biche) is 250 km south of the surface mineable area (SMA; Fort McMurray, Alberta, Canada), Burnt Lakes is 140 km west of the SMA, and Saline Lake is located in the centre of the SMA.

Table 2-1 *Select physical (Atlas of Alberta Lakes) and chemical measures for 4 study freshwater lakes, including Lac La Biche, Saline Lake, Lake Athabasca, and Burnt Lakes, Alberta, Canada. Sediment elemental analysis data are also presented.*

	Site			
Parameter	**Lac La Biche**	**Saline Lake**	**Lake Athabasca**	**Burnt Lakes**
Latitude (DD)	54.876561	57.080561	58.802363	57.266097
Longitude (DD)	-112.122386	-111.521663	-110.52502	-113.939576
Drainage Basin	Athabasca River Basin	Athabasca River Basin	Lake Athabasca Basin	Peace River Basin
Surface Area (km^2)	234	1.7	7770	3.2
Max Depth (m)	21.3	<3	124	N/A
Hardness (as CaCO3 – mg/L)	128	176	33	56
Alkalinity (CaCO3) to pH 4.5 (mg/L)	143	98	31	54
Conductivity @ 25^oC (µmho/cm)	275	3260	79	113
pH @ 25^oC	8.07	8.96	7.14	7.39
Colour (TCU)	11	15	9	12
Turbidity (NTU)	1.4	0.5	7.3	0.6
Fluoride (mg/L)	0.2	0.3	0.2	0.1
Chloride (mg/L)	3.4	990	3.3	0.5
Nitrite (N; mg/L)	< 0.1	< 0.1	< 0.1	< 0.1
Nitrate (N; mg/L)	0.1	< 0.1	0.1	0.4
Sulphate (mg/L)	5	94	5	3
Calcium (mg/L)	32.9	39.1	8.9	15.5
Magnesium (mg/L)	11.2	19	2.58	4.19

Table 2-1 continued

Sodium (mg/L)	12.4	586	3.1	1.9
Potassium (mg/L)	2.6	2.1	0.9	0.6
Copper (mg/L)	< 0.002	< 0.002	0.003	< 0.002
Iron (mg/L)	0.008	0.011	0.308	0.089
Manganese (mg/L)	0.127	0.018	0.026	0.006
Zinc (mg/L)	< 0.005	< 0.005	< 0.005	< 0.005
Ammonia (N) – total (mg/L)	0.29	0.02	< 0.01	0.06
o-Phosphate (P; mg/L)	0.16	< 0.01	< 0.01	< 0.01
Sediment Elemental Analysis				
%N (average ± SD)	1.95 ± 0.18	0.53 ± 0.23	0.13 ± 0.01	1.87 ± 0.5
%C (average ± SD)	14.83 ± 1.11	9.85 ± 3.44	2.36 ± 0.16	16.85 ± 3.85
%H (average ± SD)	2.47 ± 0.16	1.1 ± 0.18	0.75 ± 0.08	2.55 ± 0.59
%S (average ± SD)	0.41 ± 0.1	1.02 ± 0.35	0.11 ± 0.04	0.21 ± 0.05

2.3.2. Field Sampling

Surface water grabs (0.5m) were collected from the lake centre by boat, using nitrile gloves and chemically-treated (soaked in HCl for 3 days followed by triple rinsing with deionized water) 1L polyethylene bottles. Bottles were triple rinsed with lake water and filled to the rim to void the sample of air. Water samples were kept cool (4°C) and in the dark until analysis at Caduceon Laboratories, a CALA accredited ISO/IEC 17025 certified facility (Ottawa, Ontario, Canada).

Sediment cores were subsequently collected using a Uwitec gravity corer (Uwitec, Austria) with a 60cm x 8.6cm PVC tube. Sediment cores were then sectioned in 0.5 cm slices, and frozen in Whirlpack© bags at -20 ^{o}C until radiometric dating and chemical analysis.

2.3.3. Sediment Analysis

Radioisotope Measures

Sediment core chronology was determined with ^{210}Pb, ^{137}Cs, and ^{226}Ra activities measured on an Ortec High Purity Germanium Gamma Spectrometer (Oak Ridge, TN, USA). Certified Reference Materials obtained from International Atomic Energy Association (Vienna, Austria) were used for efficiency corrections, and results were analyzed using ScienTissiME (Barry's Bay, ON, Canada). Dates were determined using the constant rate of supply model for all lakes except Lake Athabasca, for which a constant flux, constant sedimentation rate (CFCS) model was selected. The agreement of the calculated date for 1963 and the observed ^{137}Cs maximum concentration in the core for Lake Athabasca validated the CFCS model.

PAC Analyses and Dosing Solutions

25 g wet sediment samples were homogenized with diatomaceous earth (Agilent Technologies, Santa Clara, CA, USA) and extracted with dichloromethane (DCM) via accelerated solvent extraction (ASE Dionex 200; at 100 °C and 1500 psi, 5 min heating time, 5 min static time and 3 cycles). Extracts underwent liquid-liquid extraction with additional DCM and were run through a sodium sulfate bed to remove any residual water,

then were further cleaned following US EPA Method 3640A modified for Envirogel™ columns (Waters Corporation, Milford, MA, USA). PACs were fractionated using a 6 g silica gel column (Grade 923, 100 – 200 mesh, EMD Millipore, Billerica, MA, USA) adapted from [42]. PACs were analysed by gas-chromatography (Agilent 6890) – mass spectrometry (Agilent 5973) (GC-MS) and quantified via a 5 point external standard curve. Injections were made in pulsed splitless mode at 280 °C on a DB-5MS 30 m x 0.25 µm x 250 µm column. An initial oven temperature of 60 °C was held for 2 min, then increased at a rate of 6 °C per minute to 300 °C and held for 4 min. A constant flow rate of 35 cm per second of helium was used for a total run time of 46 min. The mass spectrometer was set to have a transfer line temperature of 310 °C, with a source temperature of 230 °C and quadrupole temperature of 150 °C. Method blanks of diatomaceous earth (n = 7) were spiked with known concentrations of ^{13}C-labeled PACs (Cambridge Isotope Laboratories, Tewksbury, MA, USA) and extracted following the same procedures. Mean PAC recovery rates are presented in Table S1 and were used to recovery correct sample analytes after method blank corrections. Post analysis and quantification, samples were solvent switched into 1 mL dimethyl sulfoxide (DMSO) for Chemical Activated LUciferase gene eXpression (CALUX) exposure experiments.

2.3.4. CALUX Bioassays

ERα-CALUX

A human bone osteosarcoma epithelial cell line (U2OS Line) was singly transfected with a luciferase reporter gene designed to selectively measure the receptor-mediated activity of estrogen receptor α (ERα). The U2OS cell line was transfected with 3x ERE-TATA-Luc

and pSG5-neo-hERα at BioDetection System Laboratories (Amsterdam, Netherlands), where the battery of CALUX assays used in this project were developed and run. The ERα CALUX cells were plated in 96-well plates at approx. 6,000 cells per well with phenol red-free DF medium supplemented with 5% dextran-coated charcoal-stripped fetal calf serum (FCS) at a volume of 200 µL per well. After 48 hours, the medium was refreshed and the cells were incubated with the sediment core extracts (dissolved in 1% DMSO) in triplicate at a 1:1000 dilution. After 24h incubation, media was removed and cells were washed with 100µL PBS and then lysed in 30µL of Triton-lysis buffer. The preparation of the sediment core extracts as well as the exposure of the cells was performed on a Hamilton Starlet liquid handling robot coupled to a Cytomat incubator. Luciferase was measured using a CentroXS LB90 luminometer (Berthold Technologies) and values were expressed as 17β-estradiol equivalents.

PAH-CALUX

Stably transfected to contain 4xDRE (dioxin-response element) in front of a minimal adenovirus E1B TATA promoter and the luciferase reporter gene, a rat hepatoma (H4IIE) cell line was cultured in α-MEM medium supplemented with 10% FCS as outlined in [43]. Cells were plated in 96-well cell culture plates in the same growth medium at a volume of 100 µL per well (approx. 40,000 cells/well). An additional 100 µL of growth medium containing the PAC extract (dissolved in DMSO) from each core section was added to the cells in triplicates (final in well DMSO concentration was 0.8%). In parallel, a concentration series representing a full response curve of benzo(a)pyrene (BaP) was added to each plate (also in triplicate). After 4h, the media was removed, cells were washed with 100 µL PBS

and lysed in 30 µL of Triton-lysis buffer. Luciferase activity was measured using a CentroXS LB90 luminometer (Berthold Technologies) and benchmarked against the BaP treatment.

2.3.5. Data Analysis

To evaluate the difference in PAC composition both within site (n=8-11) and between sites (n=4) we used a principal component analysis (PCA) using the percentage of each PAC (n=56). First, a PCA was conducted on the whole dataset to determine the variance structure of the data related to site. This analysis was then followed up with a PCA on each lake to quantify the variance structure of PAC composition related to pre- and post-oil sands development. The amount of variance attributed to site and oil sands development was further quantified using a between-class analysis (BCA). The PCA and BCA was conducted using the stats and ade4 packages in R [44,45]. The results were visualized using scree plots and biplots which were generated using the library FactoMineR [46]. In the biplots, only PAC variables that performed better than random chance are displayed. Finally, the chronologically constrained enrichment of PACs in each lake was determined using a Mann-Kendall trend test where appropriate.

We also used three diagnostic ratios that have been validated in the environmental context of the oil sand to assess the source of PACs measured in the sediment cores. These diagnostic ratios are based on the assumption that compounds of similar molecular weight and structure have similar physico-chemical properties that influence their transport, availability, uptake and degradation in the environment. Diagnostic ratios are becoming increasingly used to determine point source emissions [47,48]. These included

indeno[1,2,3-cd]pyrene/(indeno[1,2,3-c,d]pyrene + benzo[g,h,i]perylene) or IcdP/(IcdP + BghiP); fluoranthene/fluoranthene + pyrene or Fl/Fl+Py and finally, the pyrogenic index calculated as described in [48].

Results of the PAH-CALUX were expressed in benzo(α)pyrene (BaP) equivalent quotients (bioTEQ, or BioBaPeq) because the bioassay quantifies the AhR inducing potential of the environmental PAC mixture in relation to the AhR inducing potential of a reference compound, or in this case, BaP (similar to benchmarking the ERα-CALUX data against the response obtained from 17β estradiol). As such, the results for the PAH-CALUX can be compared to those generated by chemical analysis on an instrument such as a GC-MS (with subsequent chemTEQs, or ChemBaPeq). ChemBaPeq values are calculated by multiplying concentrations of all single compounds in the dosing extract with their specific toxicity equivalent factors (TEFs). TEF values are the AhR inducing potential of a single compound to that of BaP, which has a TEF of 1. These World Health Organization (WHO) accepted TEF values were derived using careful scientific judgement while considering all available scientific data, and were compiled from [49] and [50]. By summing the calculated TEQs of all individual compounds detected through chemical analysis on a GC-MS the overall ChemBaPeq value was calculated. These values are expressed in ng BaPeq/g organic carbon in our sediment samples. For example, a value of 2 ng BaPeq/g organic carbon would mean that 1 g of organic carbon in that sediment sample would have the same effect as 2 ng of BaP.

2.4. Results

2.4.1. Historical Trends in PAC Deposition in the AOSR

Due to their high affinity for sedimentary organic carbon, PAC sediment concentrations were normalized to the total organic carbon (TOC) levels in each sediment sample, however, dry weight normalized levels are also reported in Figure S1. Alkylated compounds dominated the sum PAC contaminant signatures at all sites. The sediment of Burnt Lakes (140 kms west of the SMA) and Lac La Biche (250 kms south of the SMA) were the lowest in PAC concentration (Figure 2-1). ∑ParentPAC compounds at Burnt Lakes ranged from 389 - 2007 ng/g total organic carbon (TOC) while Lac La Biche ranged between 1455 – 3175 ng/g TOC. ∑AlkylatedPAC levels at Burnt Lakes were measured between 723 – 5899 ng/g TOC and 2443 – 7023 ng/g TOC at Lac La Biche (Figure 2-1). PAC sediment levels were the highest in the surface mineable area (SMA, Saline Lake), and downstream of the oil sands at the mouth of the Athabasca River in Lake Athabasca. At these sites, ∑ParentPAC compounds were measured at levels ranging from 2205 - 9456 ng/g TOC and 7058 - 18623 ng/g TOC respectively. ∑AlkylatedPAC levels at Saline Lake in the SMA ranged from 9893 – 48100 ng/g TOC and 21728 – 58993 ng/g TOC downstream of the SMA in Lake Athabasca (Figure 2-1).

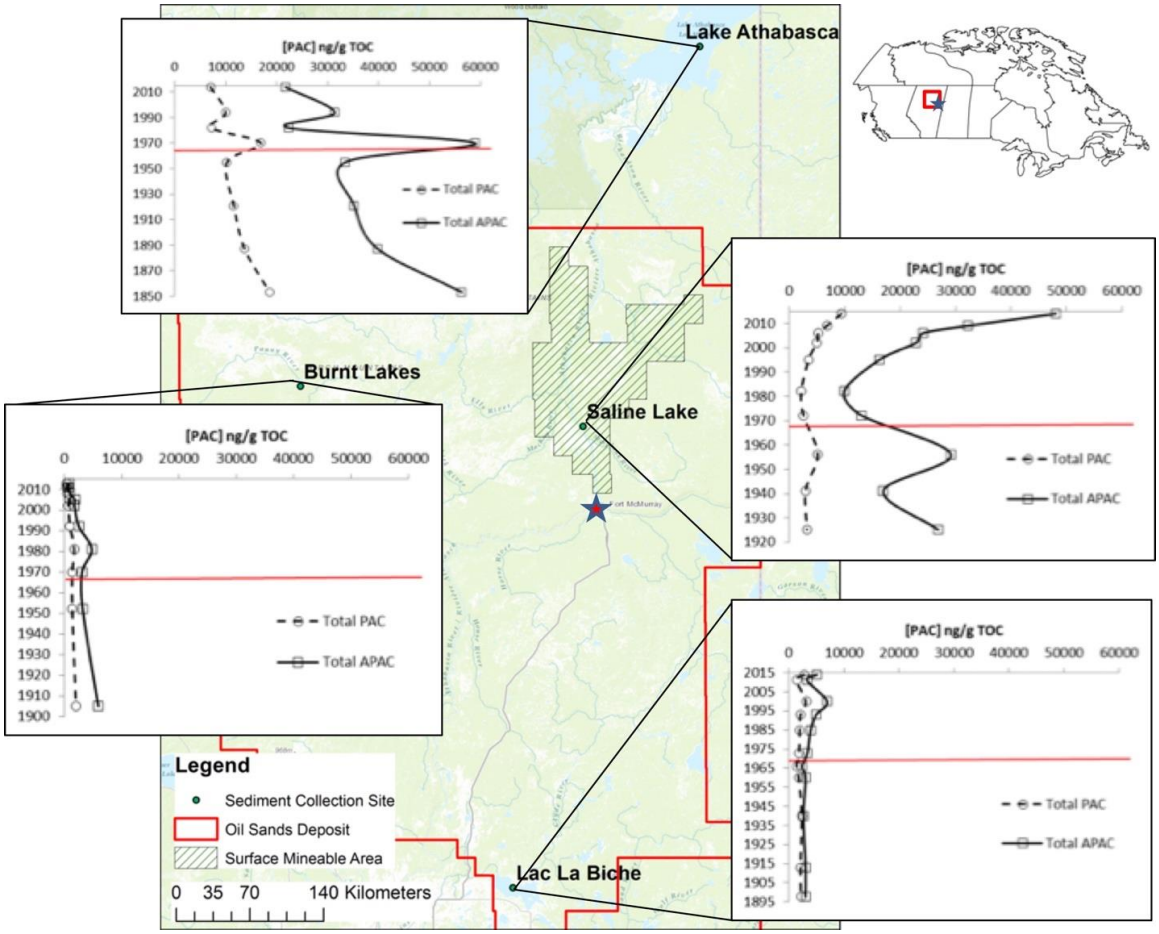

Figure 2-1 *Paleolimnological results of polycyclic aromatic compounds (PACs) and their alkylated congeners (APACs) in sediment cores collected at four sites in Alberta, Canada. Concentration results are standardized based on total organic carbon (TOC). Fort McMurray (star), surface mineable area (SMA; green cross hatched polygon) and the Alberta Oil Sands Region (AOSR; red outline) are also presented.*

Background (pre-oil sands development – i.e. <1967) PAC and alkylated PAC (APAC) were variable at all sites. Although there was evidence of an increase in PAC and APAC in the uppermost 2 sediment intervals (i.e. post development) at Lac La Biche and Saline Lake (Figure 2-2), the Mann-Kendall trend tests revealed that this enrichment was only

significant for APACs at Lac La Biche (p=0.02) and for PACs at Saline Lake (p=0.03). There were no trends detected in the series of the other compounds at these two sites. Conversely, significant decreasing trends in PAC and APAC concentrations were detected in the Lake Athabasca sediment core (p=0.02 and p=0.04 respectively) and the sediment of Burnt Lakes (p=0.002 and p=0.001 respectively). Ranking in total recent PAC levels between sites was Saline Lake> Lake Athabasca> Lac La Biche> Burnt Lakes.

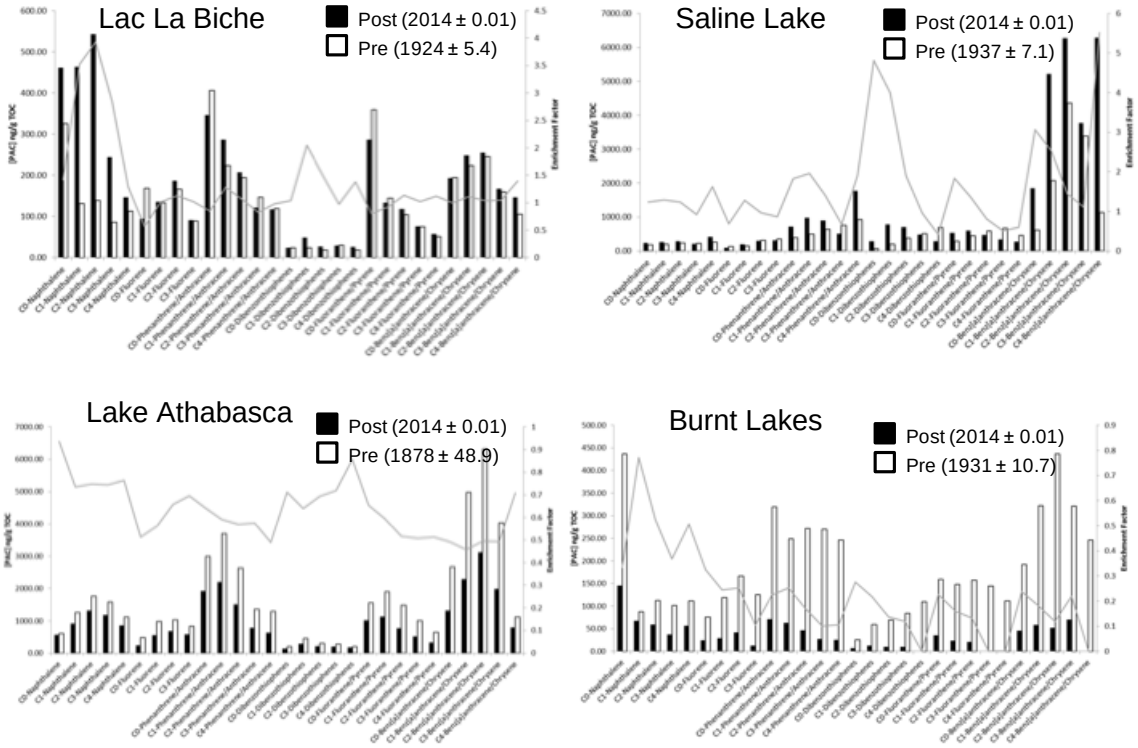

Figure 2-2 *Average PAC and APAC concentrations in top 2 (post-industrial) and bottom 2 (pre-industrial) sediment intervals in sediment cores. Enrichment factors (line) are presented for each compound class, and plotted against secondary Y-axis.*

The PCA results on the PAC composition between sites show that a large proportion of the variance in composition is related to the site (Figure 2-3). In components 1 and 2, as well as 3 and 4, all lakes show distinct clustering on the biplot. In the first two components, which account for 76.6% of the variance in the data, Lac La Biche, Burnt Lake, and Lake Athabasca were more similar in PAC composition than Saline Lake. The composition of PACs in Saline Lake are dominated by the benz(a)anthracene/chrysene and benzofluoranthene/benzopyrene series. The composition of PACs in Lac La Biche, Burnt Lake, and Lake Athabasca are dominated by the naphthalene and phenanthrene series. These compounds are also highly loaded in components 3 and 4, which cumulatively explains 90.9% of the variance in the data.

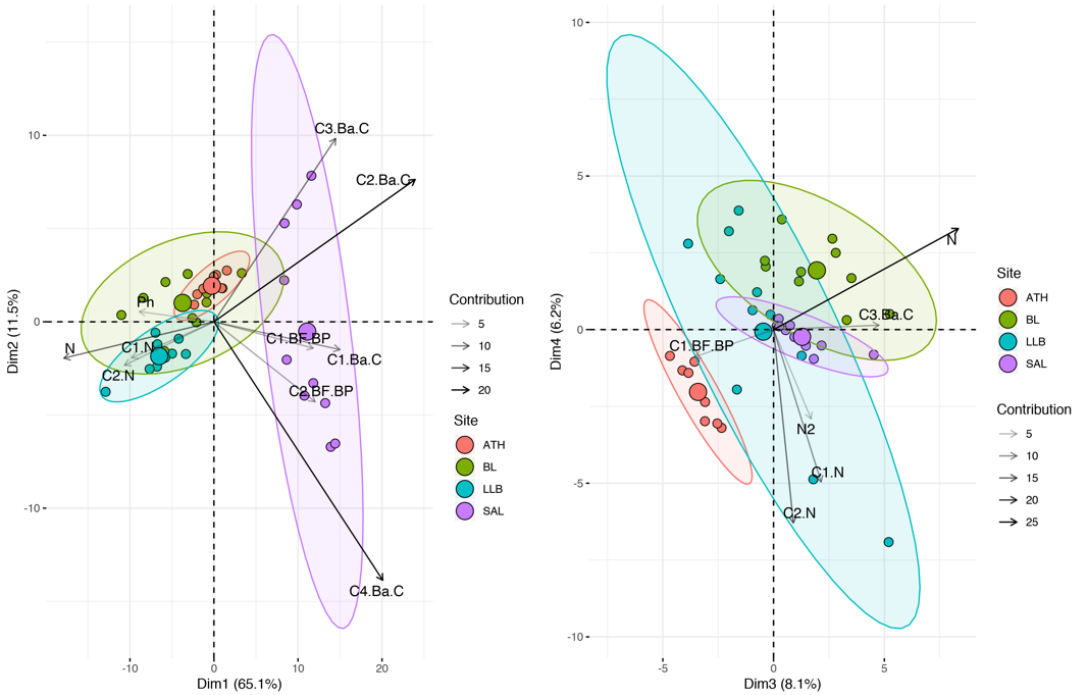

Figure 2-3 *Principal component analysis (PCA) of PAC composition on the combined dataset showing clustering of the cases (i.e. sediment interval). The large circle represents the center of the cluster, and the ellipse represents the 95% confidence interval of the cluster. The arrows represent the variables (PACs) that contribute a relatively large proportion to the total distance. The darker and larger the arrows the more variance is explained by that variable. ATH= Lake Athabasca, BL= Burnt Lakes, LLB= Lac La Biche, SAL= Saline Lake*

The PCA results on the PAC composition within sites show oil sands development has impacted the PAC composition in the lakes over time (Figure 2-4). The clustering of PAC composition pre and post oil sands development is more prominent in the first two components at Lac La Biche, Burnt Lake, and Saline Lake (Figure 2-4). Conversely, at Lake Athabasca there is no distinct pattern in PAC composition related to the onset of development. This is also reflected in the BCA results as only 5% (p=0.057) of the variance in the data can be attributed to the onset of mining. At Lac La Biche, the naphthalene

series were significantly enriched in recent sediment post-development. While at Saline Lake and Burnt Lake, the benzo(α)anthracene/chrysene and benzofluoranthene/ benzopyrene series (including both parent and alkylated congeners) were enriched post-development. While there is no distinct pre and post development clustering for Lake Athabasca, this PCA is dominated by the naphthalene, benzo(α)anthracene/chrysene and benzofluoranthene/ benzopyrene series. The results of the PCA are also in accordance with the results presented in Figure 2-2, where the average top 2 sediment intervals (post-development) were compared to the average of the bottom 2 sediment intervals (pre-development).

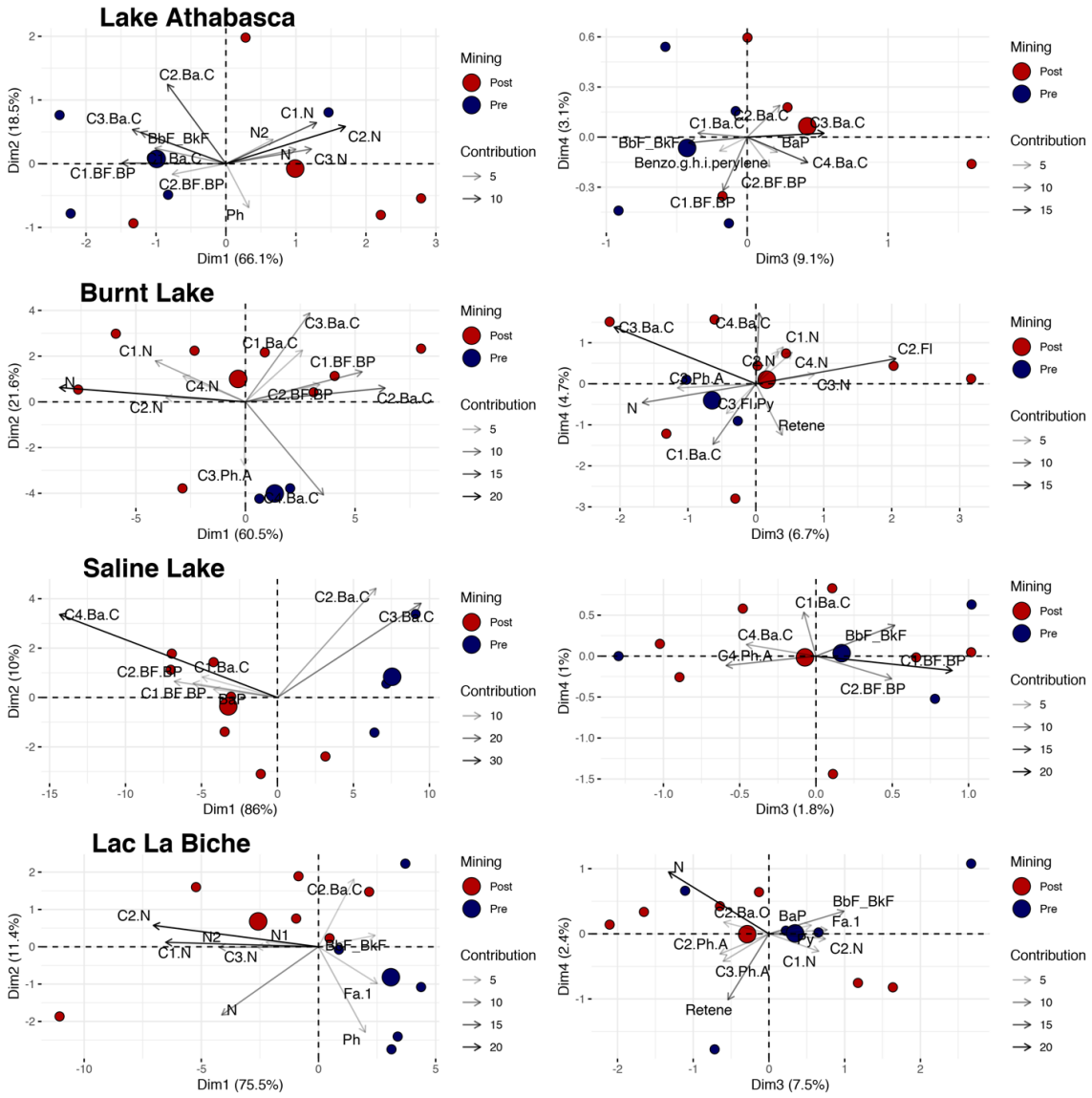

Figure 2-4 *Principal component analysis (PCA) of PAC composition on the combined dataset showing clustering of the cases (i.e. sediment interval) pre (blue) and post (red) mining development. The large circle represents the center of the cluster. The arrows represent the variables (PACs) that contribute a relatively large proportion to the total distance. The darker and larger the arrows the more variance is explained by that variable.*

Further, there are notable temporal and compositional differences in PAC/APACs between the different sites in agreement with the results of the Mann-Kendall trend tests. Lower molecular weight petrogenic PACs weighed heaviest on the compositional relationship at Lac La Biche and Saline Lake in the SMA while higher molecular weight pyrogenic PACs exerted the strongest influence at Burnt Lakes and Lake Athabasca. The Mann-Kendall trend results are also in agreement with the PAC concentration down core found in Figure 2-1, where pre-oil sands development PAC levels in Lake Athabasca are higher than post-development levels presented (Figure 2-2).

Potential sources of PACs in aquatic sediment cores

Even though limitations in discriminating between different sources are well recognized, PAC diagnostic ratios remain good qualitative tools that can help inform likely sources of PAC enrichment [47]. Figure 2-5 presents the diagnostic ratio values calculated down core. At Lac La Biche, PACs were mostly of pyrogenic origin (on the right side of the green line) except for a brief petrogenic input in recent (~2000) sediment, consistent with the naphthalene series input detected above. Saline Lake sediment were mostly influenced by petrogenic PACs (on the left side of the green line) with a recent increase in the proportion of PACs of pyrogenic origin (Figure 2-5). The same pattern was detected in Lake Athabasca, while PACs at Burnt Lakes shifted from a proportionately dominated pyrogenic signature to a more petrogenic signature post-development.

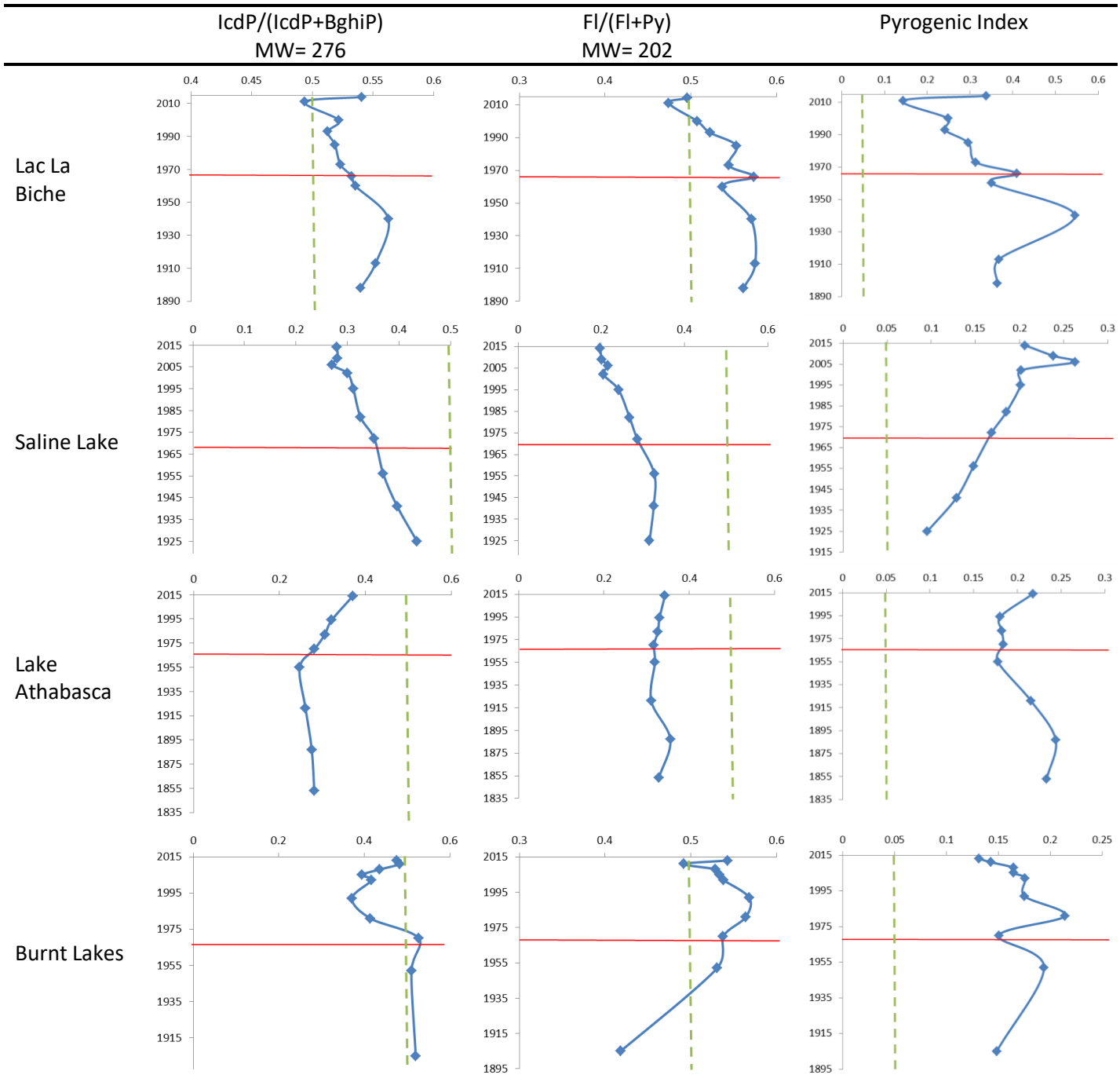

Figure 2-5 *Results of three diagnostic ratios in sediment cores of our four sites are presented, including indeno[1,2,3-cd]pyrene/(indeno[1,2,3-c,d]pyrene + benzo[g,h,i]perylene) or IcdP/(IcdP + BghiP); fluoranthene/fluoranthene + pyrene or Fl/Fl+Py and finally, the pyrogenic index calculated as described in (45). Petrogenic (left side of the green line) and pyrogenic (right side of the green line) signatures are presented down core.*

2.4.2. Chronologically-constrained biological response to environmental PAC mixtures

ERα-CALUX assays

Estrogen receptor mediated signaling was successfully measured in a mammalian cell-line after exposure to PAC sediment extracts (Figure 2-6). ERα-mediated signaling was variable down core at all four sites with no clear trend in activity detected. However, a clear increase in estrogen receptor activity was measured around 2010 at every site but Lake Athabasca. Sediment extracts from Lake Athabasca elicited the strongest estrogen response early in the 19th century when PAC levels were also at their highest.

We detected ERα responses in sediment cores from all sites, ranked as Saline Lake> Burnt Lakes> Lake Athabasca> Lac La Biche (2.44 >1.67 >1.57> 0.88 ng 17β estradiol/g organic carbon). However, there were no relationships detected through linear regression analyses between the ERα-CALUX signal and any of the individual PACs measured in the dosing extracts, the sum of these compounds (i.e. parent and alkylated congeners combined), their diagnostic ratios or any of the factor scores calculated during the principal component analysis.

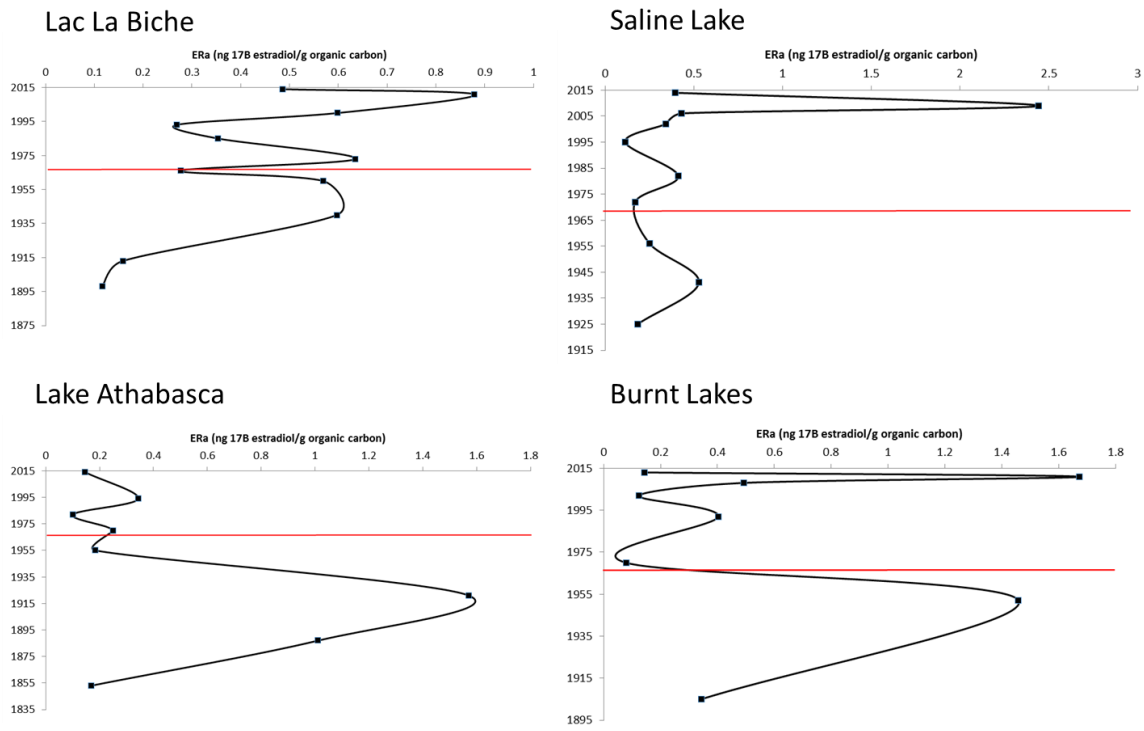

Figure 2-6 *Down core profile of estrogen receptor alpha (ERα)-mediated signaling obtained through Chemically Activated LUciferase eXpression (CALUX) bioassay of PAC extract exposure in a U2OS cell line. Results of the four sites are presented and contrasted.*

PAH-CALUX assays and toxicity equivalent quotients (TEQs)

PAH-CALUX assay results complemented those of the chemical analysis in Figure 2-1. In fact, the trends of the chemical profiles of parent and alkylated PACs almost mirrored those of the PAH-CALUX assay (Figure 2-7: middle column).

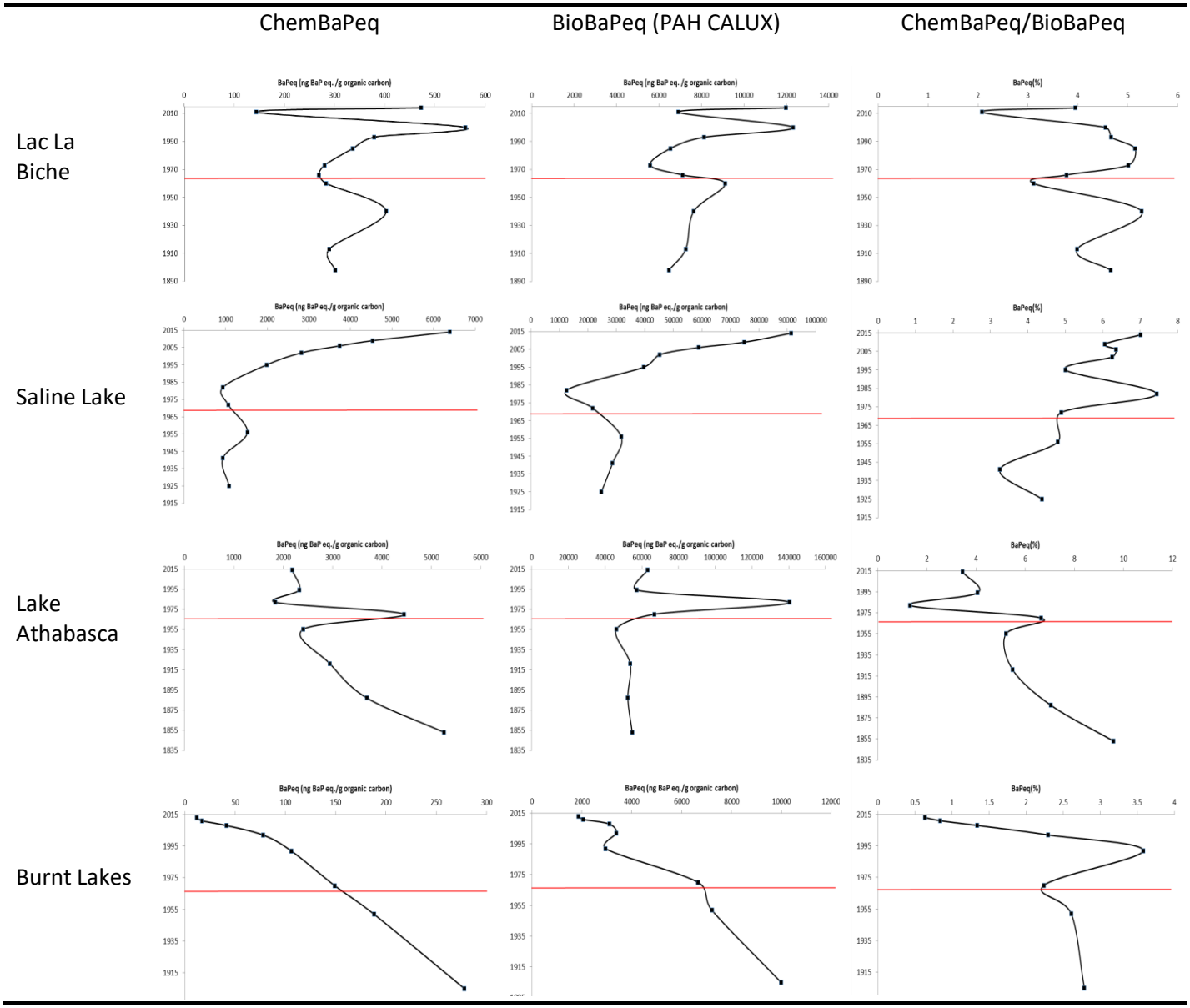

Figure 2-7 *Down core profile of PAH-CALUX expressed as benzo(α)pyrene (BaP) equivalent quotients (bioTEQ, or BioBaPeq). ChemBaPeq values obtained through GC-MS analysis, and the ratio of ChemBaPeq/BioBaPeq are also presented as an index of how well we are characterizing AhR agonists.*

Sediment from Saline Lake had the highest ChemBaPeq values followed by Lake Athabasca, Lac La Biche, and Burnt Lakes (6392>5257>472>278 ng BaPeq/g organic carbon respectively; Figure 2-7). ChemTEQ values in Burnt Lakes and Lake Athabasca

significantly decreased from pre-development times (Mann-Kendall trend test; p=0.001 and p=0.035 respectively), increased since pre-development at Saline Lake (Mann-Kendall trend test; p=0.004) while no temporal trends were detected in Lac La Biche (Mann-Kendall trend test; p=0.64). It is worth noting that the underlying effect levels of the TEQs were the EC50.

2.5. Discussion

This is the first study to successfully reconstruct hydrocarbon deposition to aquatic sediments in the AOSR while providing a framework on how sediment core extracts can be used to contextualize the biological significance of these historical changes using a paleotoxicological approach.

2.5.1. Spatial and temporal PAC deposition trends to aquatic sediments

We investigated not only historical and compositional patterns in PAC deposition to aquatic sediments in the AOSR, but we also included a wider regional context by providing reference data at more distant, less impacted sites (i.e., Burnt Lakes). The chronologically-constrained PAC data generated in our sediment cores can be interpreted through a structured and adaptive framework such as the Canadian Environmental Effects Monitoring (EEM) program [51]. Such frameworks allow us to further contextualize the relevance of changes detected in PAC levels at our four sites.

In the context of EEM, given declining trends in PACs in aquatic sediments of Lake Athabasca and Burnt Lakes and further considering that none of the measured PACs

exceeded currently accepted sediment quality guidelines (CCME), there is no justification for a deeper analysis. Conversely, sediment in Lac La Biche (south of the SMA) and Saline Lake (in the SMA) showed opposite trends to Burnt Lakes and Lake Athabasca, with increasing levels of APACs and PACs respectively. Thus, applying a monitoring interpretation framework (EEM) to the data at Lac La Biche and Saline Lake allows us to further evaluate the significance of these changes.

Levels in recent sediment at Lac La Biche (upstream the SMA) were orders of magnitude lower than the ones measured at Saline Lake in the centre of the SMA. The detection of elevated APACs in the Lac La Biche core above the 95% confidence interval of the mean baseline (i.e. the average of the 6 deepest sediment samples dated <1973) appeared to confirm a change that was unique to this site. A continued increase in APACs above the trigger limit of the mean baseline ± 2*SD appeared to confirm the extent of this change, peaking in ~2000 before only recently returning to baseline levels in 2011 (i.e. under trigger limits, and closer to 95% confidence interval). Interim sediment quality guidelines (CCME) were exceeded in 4 lower molecular weight PACs (including naphthalene and its alkylated congener 2-methylnaphthalene, acenaphthene and phenanthrene), but did not reach probable effects levels even at their peak in 2000. Our results suggest that the level of PACs necessary for causing ecological change would need to be higher than what we currently observed. A study by Munkittrick and Arciszewski (2017) synthesized sediment core data in the AOSR and determined that APAC enrichments were not likely to occur naturally, so it is probable that the change to Lac La Biche was driven by something other than natural variability [51].

Similar to Korosi et al. (2016), we detect an increase in APAC, but not parent compounds is an indication of a petrogenic input as APACs are more abundant than parent compounds in petroleum products [52]. Two diagnostic ratios and the pyrogenic index provide further evidence of a recent petrogenic input to Lac La Biche. Furthermore, the fact that the dibenzothiophene series were significantly enriched (second after lower molecular weight naphthalenes) is indication of a petrogenic influence as the occurrence of these sulfur-containing compounds in environmental media has been attributed to oil sands development [18,20,53]. Together, this weight of evidence suggests a recent input of petrogenic PACs to Lac La Biche. Korosi et al. (2016) provided evidence linking increases in C2-naphthalene in lake sediment to bitumen flow-to-surface (FTS) events where cyclic steam stimulation (CSS) operations during in-situ extractions contribute to the vertical release of bitumen to surface or aquatic environments. Our results also support these findings with C2-naphthalenes being most enriched in recent sediment and declining back to background levels, leading us to conclude that an FTS event might have impacted Lac La Biche early in the 21st century (~2000).

Levels of PACs were highest at Saline Lake in the SMA and under an EEM framework, this site would justify proceeding to an investigation of cause and effect. The detection of elevated unsubstituted PACs in the Saline Lake core above the 95% confidence interval of the mean baseline (i.e. the average of the 4 deepest sediment samples dated <1972; before significant oil sands development in the region) appeared to confirm a change that was unique to this site as well. A gradual and sustained increase in the levels of PACs above the trigger limit of the mean baseline ± 2*SD appeared to confirm the extent and

likely magnitude of this increase. Unlike Lac La Biche, this increase is sustained and continues to increase. Interim sediment quality guidelines (CCME) were exceeded in the majority (9 out of 13) of compounds where sediment quality guidelines exists, from lower molecular weight petrogenic PACs to higher molecular weight pyrogenic compounds such as the highly toxic benzo(a)pyrene (BaP). In fact, BaP more frequently reached levels approaching probable effects levels when compared with levels of all other compounds. Providing biological context to these data and understanding the significance of these changes would thus become important, and a paleotoxicological approach can contribute to this understanding.

The levels reported in our Saline Lake core east of the Athabasca River (~5km downwind major oil sands upgrading facilities) are similar to those reported in Kurek et al. (2013). The compositional makeup of the detected PACs are similar to those reported in snow and other sediment cores in the AOSR with a greater contribution of high molecular weight PACs such as benzo(a)anthracene and benzo(a)pyrene as well as APACs and DBTs [19,53]. PAC and APAC levels in the Saline Lake core were also higher than those reported in the upper sediment intervals from Albertan lakes within tens of kilometers of coal-fired power plants and extensive agricultural and residential developments [54]. Comparatively, Lac La Biche, Lake Athabasca, and Burnt Lakes were within the range of PAC values typical of remote lakes in Alberta, which is substantially lower than lakes in other urbanized catchments (compiled in [20]).

2.5.2. PAC Sources and Historical Changes

The PAC composition in Lake Athabasca, Lac La Biche, and Burnt Lake remained distinct from the one measured at Saline Lake as represented by the divergent 95% confidence interval ellipse in Figure 3. This is especially true throughout the recent past (i.e. top 5cm of the sediment core). Individual PCAs for each lake revealed a directional change in PAC composition at each site post oil sands development. The lower molecular weight naphthalene series weighed most heavily on component 1 and 2 at Lac La Biche and Burnt Lakes (Figure 4). Saline Lake sediment were mostly enriched in alkylated naphthalenes, but with the added influence of alkylated benzo(a)anthracene/chrysene .

2.5.3. Historical Reconstruction of Biological Effects

Our sediment cores were used to reconstructing historical patterns in PAC deposition to aquatic sediment establishing concentrations which could have caused adverse biological effects. To further investigate biological effects we also shown how these samples can be used in bioassays, such as the CALUX assays, to determine how historical PAC extracts can disrupt endocrine processes. The bioassay-derived data is useful in contextualizing the potential biological relevance of changes in PAC deposition. By including a remote reference site (i.e. Burnt Lakes), this can help determine whether biological changes in areas impacted by oil sands activity are different than “background” at the remote site.

The *in vitro* CALUX assays that were used in our study were based on mammalian cell lines that were genetically modified to produce luciferase after exposure to samples that

evoke a biological response. The effect is quantified by comparing that response to the signal measured after exposure to a reference compound. However, a limitation of the U2OS cell line used in the ERαCALUX assay is that there is minimal to no expression of other t nuclear receptors. Without this it is not possible to identify crosstalk between nuclear receptor-mediated activities which may in turn omit important EDC biological activity [55,56]. Furthermore, several review papers have been published indicating that metabolism is particularly important when testing EDCs, since several hormonally active chemicals are known to be subject to metabolism [57,58]. Since compounds can be converted into either more active or less active metabolites [59], neglecting to determine metabolism can lead to both false negatives and false positives [60]. The ERα CALUX system has been shown to have very low basal metabolic capacity which could have indirectly affected PAC metabolite levels and therefore subsequent luciferase activity [60]. These facts might in fact explain the lack of any spatial or temporal pattern in our ERα activity measured in the sediment of our four sites. Future ERα-CALUX work should include a metabolically active module such as rat liver S9 to mimic in vivo hepatic metabolism and downstream endocrine disrupting potential.

The PAH-CALUX bioassay provided some spatial and temporal information on PAC burdens to aquatic sediments that paralleled the chemical analysis. By assessing at the ratio of ChemBaPeq/BioBaPeq, we can determine how accurately we can quantify suspected AhR agonists (such as PACs) in complex environmental samples. Since PAH-CALUX assays integrate the overall gene activating effect of all AhR agonists and antagonists present in a chemical mixture, it is possible to determine whether or not the

chemical analyses are effective at pinpointing the actual chemicals responsible for a biological effect by calculating this ratio. [30] The closer the calculated metric is to one (or 100 when multiplied by this factor), the better our chemical analyses are at pinpointing causative agents. At the 4 sites, the results of our chemical analyses accounted for less than 10% of the overall AhR activation potential of the mixture (Figure 7 – right column). At Burnt Lakes, less than 1% of the overall AhR inducing compounds present in the mixture were targeted while at Saline Lake and Lake Athabasca, our chemical analyses quantified up to 7% and 10% of those compounds respectively. Future analytical chemistry work should focus on identifying novel PAC compounds in sediment extracts that could be important drivers of toxicity.

2.6. Conclusions

Paleolimnology and the study of sediment cores continues to be an important and powerful tool in measuring temporal variation in PAC deposition to aquatic sediments. As a monitoring tool, sediment cores are advantageous as they are not dependent on daily inputs which is a limitation to contextualizing the biological significance of daily air or water fluctuations, for example. The biological signal measured through CALUX assays in this study was chronologically constrained in each sediment interval. By broadening the geographical extent of sampling, a better understanding of spatial and temporal patterns related to chemical exposures and resulting biological effects can be better quantified. This is useful when positively identifying input events which may not have been reported, or underreported, as is the case with accidental oil spills or flow to surface events. A

paleotoxicological framework (where paleolimnology includes measures of biological response) are useful in contextualizing the biological significance of these changes. This study shows that there are spatial and temporal variability in the PAC composition of sediment cores around the AOSR. Our study is the first to link chemical analysis of sediment cores with endocrine function showing feasibility of extending the usefulness of sediment cores in monitoring programs. While we observed no spatial or temporal differences in ERα mediated signaling, this approach demonstrates the powerful potential of coupling biological effects assessments to historical reconstructions of contaminant inputs to the natural environment. Future studies should consider using other bioassays that enable quantification of interactions between multiple nuclear-receptor signaling pathways.

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Chapter 3.

Trophodynamics of Polycyclic Aromatic Compounds (PACs) Measured in a Food Chain Dominated by River Otters (*Lontra canadensis*) in the Athabasca Oil Sands Region

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3.1. Abstract

The western Canadian sedimentary basin contains about 2.3 trillion barrels of natural bitumen (thick, heavy crude oil) rich in polycyclic aromatic compounds (PACs), fueling an active oil sands natural resource extraction sector. Enrichment of PACs from multiple sources in the local environment are raising concerns about the potential toxic effects from exposure to these compounds in fish and aquatic wildlife. There are concerns that the lipophilic nature of some of these compounds could result in higher bioaccumulation and subsequent biomagnification potential with negative health consequences in higher trophic consumers. As a result, in this study, biota samples from snails, prey and predator fish, as well as river otter (*Lontra canadensis*) were analysed for a suite of PACs as well as stable isotopes to evaluate the trophodynamics of these compounds in a representative food chain. Bioaccumulation factors such as biota-sediment accumulation factors (BSAF) and trophic magnification factors (TMF) were used to evaluate the partitioning behavior of PACs in the environment and subsequent risks to biota. We demonstrated how PACs exhibited trophic dilution in a Boreal food chain dominated by river otters. Alkylated heterocyclic aromatic compounds and alkylated higher molecular weight compounds predominately underwent trophic dilution. Our results revealed that alkylated PACs were highest downstream the oil sands in the Peace Athabasca Delta followed by the surface mineable area when compared to Burnt Lakes, a lower impact study area. While we did not uncover any effect of age or sex in PAC residue levels across all biota, we did show that seasonality could be driving exposure to different PAC compounds. Our results suggest that ingestion, assimilation and subsequent depuration rates were more plausible

explanations of trophic dilution relationships detected in Athabasca oil sands region (AOSR) biota than the lipophilic nature of PACs.

3.2. Introduction

Polycyclic aromatic compounds (PACs) represent a group of aromatic organic contaminants of both petrogenic and pyrogenic origins. They are released into the environment from anthropogenic and natural sources as complex mixtures, encompassing thousands of different aromatic, alkylaromatic and heterocyclic hydrocarbons containing N, S, or O- atoms. Sixteen (16) of these have been designated as priority pollutants by the United States Environmental Protection Agency (US EPA), the US Agency for Toxic Substances and Disease Registry (ATSDR), and the European Food Safety Authority (EFSA) due to their toxicity [1–3]. PACs are also listed under Schedule 1 of the Canadian Environmental Protection Act because of their carcinogenicity. As a result of their hydrophobicity and lipophilicity, their capacity for long range transport, their toxicity to aquatic organisms, their bioaccumulation potential and their strong interactions with sedimentary organic carbon, PACs have also been included in the Convention for Long-Range Transboundary Air Pollution's (CLRTAP's) Persistent Organic Pollutants (POP) protocol [4]. During the past 25 years, there has been a substantial increase in PAC concentrations at lower trophic levels in some northern environments, especially the more polar alkyl aromatic hydrocarbons, and hetero-compounds [5].

The western Canadian sedimentary basin contains about 2.3 trillion barrels of natural bitumen (thick, heavy crude oil) rich in PACs [6]. This is equivalent to roughly 43% of the known global oil reserve, 170 billion barrels of which are economically recoverable by the province of Alberta [6]. Bitumen contains polycyclic aromatic hydrocarbons (PAHs), alkylated PAHs and dibenzothiophenes; collectively known as petrogenic bitumen-derived polycyclic aromatic compounds (PACs; [7]). There are two sources of bitumen-derived PACs to the environment, those released naturally from oil sands deposits (i.e., due to erosion) and those from bitumen extraction and upgrading (i.e., oil sands activity). Pyrogenic PACs can also be released to the environment as a by-product of the rapid, high temperature and often incomplete combustion of organic matter [8–11]. With annual bitumen production increasing, even in an environment of low oil prices, large volumes of oil-sands process-affected water (OSPW; rich in PACs) and fine tailings (the silt/clay fraction of bitumen extraction) are stored in tailings ponds [12]. Oil sands mining operators are further required to reclaim their leases through strategies including “wet landscape” approaches that involve the creation of lakes lined with fine tailings that are enriched in PACs and heterocyclic aromatic compounds, capped with layers of fresh water [12]. Concerns have been raised about the potential toxic effects from exposure to PACs in fish and aquatic wildlife in these reclaimed landscapes [12]. Because PACs have a high affinity for organic carbon, most are bound to particulates. Sediments and soils are often the final sinks for these compounds through deposition and sedimentation processes [13].

PACs are detected in tissues of invertebrates and vertebrates in ecosystems across Canada and at concentrations that vary with species, location, tissue type (including lipid content), and metabolic capacity. Free-ranging wildlife can be exposed to PACs through a variety of exposure pathways (inhalation, dermal absorption, ingestion/diet or maternal transfer). Exposure to LMW PACs occurs primarily through air and water whereas exposure to HMW PACs is predominately via ingestion [14]. PAC profiles in wildlife tissues can be assessed in light of the PAC fingerprints of the surrounding environment to help understand route and likely sources of exposure. For example, PAC levels in tree swallow muscle from the oil sands area (Alberta, Canada) was influenced by diet, air, and water, each contributing to the variation of the different PAC concentrations [15]. Conversely, in a food web in British Columbia (Canada) the pattern of PACs detected in sea otter blood was similar to what was measured in bivalves as a proxy of prey items suggesting that diet was the main source of PACs detected in otters [16]. While understanding the source(s) of exposure is important, the impact of these PACs on health outcomes is critical for risk assessment.

Concentrations of chemicals in biota at higher trophic levels can sometimes reach toxic levels through bioaccumulation, adversely affecting health with subsequent effects that reverberate across food webs [17]. Indeed, there are concerns that long-lived wildlife species could accumulate significant burdens of PACs leading to negative health outcomes [18]. The bioaccumulation of PACs is complex depending on source of exposure, molecular weight and the alkylation status. For example, although many simple PACs can be metabolized in higher vertebrates, some alkylated PACs accumulate in fish

tissues [19]. Controlled continuous exposures of fish to oil-containing sediments show that independent of PAC concentration, accumulation of lower molecular weight PACs reaches a steady state quickly, whereas higher molecular weight PACs did not tend to bioaccumulate after four months of exposure [18]. In aquatic insects from Canada's oil sands area, alkyl PACs generally had higher biota-sediment accumulation factors (BSAFs) than unsubstituted PACs [12], likely due to their more lipophilic nature than parental PACs. Kim and Kang (2019) have recently suggested that trophic magnification factors (TMF) are a robust metric to assess bioaccumulation of environmental pollutants.

This study aims to evaluate the bioaccumulation of different PACs across 4 distinctly different study areas in the Athabasca Oil Sands Region (AOSR) with different PAC sources using BSAF and TMF values in a representative aquatic food chain in the Boreal ecosystem (snails- fish-river otters). We hypothesize that high impact sites in the surface mineable area will have substantially higher BSAF and TMF values based on the locally-enriched PAC conditions in the environment.

3.3. Materials and Methods

3.3.1. Study Area

Samples were collected from four areas in northeastern Alberta, Canada (Figure 3-1). These study areas included varying degrees of anthropogenic disturbances, a number of freshwater lakes, wetlands, trap lines, and sites identified by traditional land users as being important for the practice of cultural and recreational activities (e.g.

hunting, fishing and trapping). Three areas (Lac La Biche, Saline Lake, Burnt Lakes) were located in the Athabasca Oil Sands Region (AOSR) in northeastern Alberta, Canada and one (Peace Athabasca Delta; PAD) was located downstream the Athabasca River inside Wood Buffalo National Park; a UNESCO World Heritage Site. Inside the AOSR, one area was dominated by steam-assisted gravity drainage infrastructure (SAGD; centered on Lac La Biche Lake, Alberta, Canada); 250 kms south of the surface mineable area (SMA; Fort McMurray, Alberta, Canada). Burnt Lake is 140 kms west of the SMA, and Saline Lake is located in the centre of the SMA (Figure 3-1).

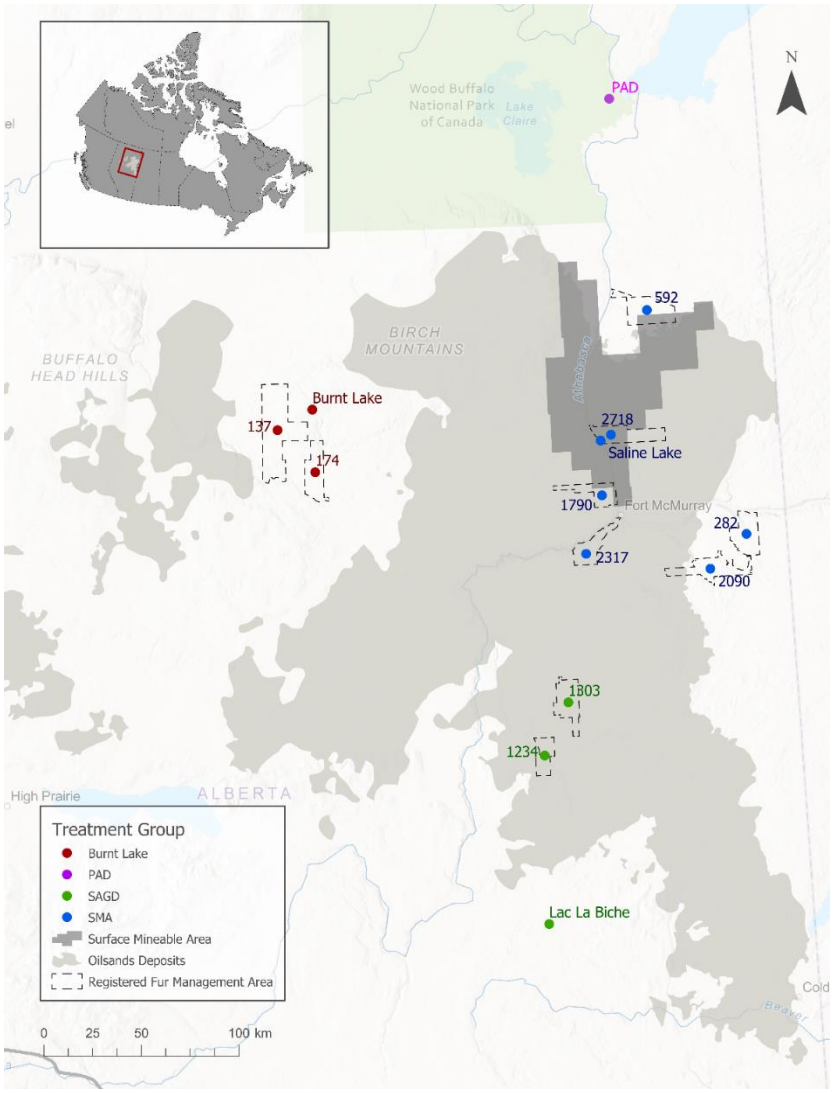

Figure 3-1 *Location of four main study areas in the Alberta Oil Sands Region (AOSR), Alberta, Canada. Registered Fur Management Area (RFMA) number as well as site name where snails and fish were collected are presented in color coded treatment groups.*

3.3.2. Sample Collections

Snails

Small-bodied snails were collected near shore submerged on sediment, or found attached to emerging vegetation. Snails were collected and placed in clean high density polyethylene (HDPE) 250 ml bottles filled with freshwater. The snails were allowed to void

their gut in freshwater for 24 hours, after which they were thoroughly rinsed in fresh water, patted dry, and frozen at -20C in clean 250 ml HDPE bottles (Fisherbrand, ThermoFisher, Ottawa, ON). Most snails were from *Physidae*, *Lymnaeidae* and *Hydrobiidae* species and all were pooled together at each site due to their small size. No snails were analyzed from the Peace Athabasca Delta (PAD).

Fish

Lake Whitefish (*Coregonus clupeaformis*), Walleye (*Sander vitreus*) and Northern Pike (*Esox lucius*) were targeted for collections. Up to 5 fish from each species were anticipated for collections at each site (n=4 sites, N=60 fish total). Sampling involved the use index gillnets. A standard index gillnet 6' deep x 200' long consisting of eight (8) monofilament panels of 1 inch to 6 inch mesh was used. Nets were set at locations selected based on the Traditional Knowledge of the Elders in the Peace Athabasca Delta and based on the local knowledge of traditional land users at Burnt Lakes, in the SMA (Saline Lake) and SAGD (Lac La Biche). Nets were set for two hours at dawn and dusk. After capture, fish were immediately sacrificed using blunt force trauma to the head. Carcasses were kept on ice for transport and subsequently frozen at -20C for toxicological analysis of hepatic PAC burdens. Animal care considerations and ethics were approved by Environment and Climate Change Canada (ACC# 1429PT03) and fish were collected on an approved provincial permit from Alberta Environment and Parks (FRL# 14-0466).

Fish were aged by removing the otoliths (ie- inner-ear bone of the fish) and counting annuli. Otoliths were shipped to AAE Tech Services (La Salle, Manitoba) for ageing using

a combination of microscopic examination, and/or sectioning using the “crack and burn” method [21]. All ageing was carried out under an image analysis system for quality control assurance.

River Otters (Lontra canadensis)

Northern Alberta commercial trappers recruited through the Alberta Trappers Association (Westlock, Alberta) assisted in the sampling of river otter samples. River otter already trapped under permit for the commercial fur trade were collected from willing trappers. All animals were trapped following the Alberta Code for Responsible Trapping and the Agreement on International Humane Trapping Standards (AIHTS). Carcasses were stored at -20°C until wildlife post-mortem evaluations and dissections were conducted at the National Wildlife Research Centre (NWRC; Ottawa, ON). Gross necropsies were undertaken on each otter. Livers were dissected and stored in chemically-cleaned amber glass jars at -20°C.

Whole liver was homogenized, and aliquots submitted for stable isotope analysis (%CN, ^{13}C, ^{15}N) at the Ján Veizer Stable Isotope Laboratory (University of Ottawa, Ottawa, ON) and polycyclic aromatic compound analysis at the Centre of Oil and Gas Research and Development (COGRAD, University of Manitoba, Winnipeg, Canada); both are ISO17025 and CALA accredited facilities.

3.3.3. Chemical Analysis

Stable Isotope Analysis

Freeze-dried, pulverized liver samples and standards were weighed into tin capsules and loaded into an elemental analyser (Vario EL Cube manufactured by Elementar, Germany) interfaced to an isotope ratio mass spectrometer (IRMS; Delta Advantage manufactured by Thermo, Germany). Sample/standard was flash combusted at ~1800°C (Dumas combustion) and the resulting gas products were carried by helium through columns of oxidizing/reducing chemicals optimized for CO_2 and N_2. The gases were separated by a purge and trap adsorption column and sent to the IRMS interface (Conflo IV, Thermo, Germany) followed by the IRMS.

Polycyclic Aromatic Compound (PAC) Analysis

The analysis of PACs and their alkylated congeners followed Idowu et al. (2018) [22]. Briefly, ~5 g of liver homogenate was accurately weighed on a Sartorius Cubis MSE225P-100-DI (Fisher Scientific, Ottawa, Canada) and mixed with diatomaceous earth (DE). The mixture was loaded in an accelerated solvent extraction (ASE) cell, spiked with ^{13}C labelled internal standards and the dead volume of the cell filled with Ottawa sand. Procedural blanks, standard reference material (NIST SRM 2974a) and duplicates were included in each sample run. Samples were extracted under high pressure conditions with dichloromethane (DCM) used as the extraction solvent. ASE conditions are outlined in Idowu et al. (2018) [22]. The extract volume was transferred to a rotary evaporator and then subjected to ultra-high purity (UHP) nitrogen blowdown to a final volume of 2.6 ml. It was then mixed in with hexane (1:1 v/v) to a final volume of 5.2ml. 200ul of this volume

was used to gravimetrically determine lipid composition. Removal of lipids from the extracts was achieved using automated gel permeation chromatography (GPC; J2-scientific AccuPrepMPS™, Columbia, Missouri, USA). The final extract was again reduced down to 1 ml under UHP nitrogen, and loaded on a silica/alumina column for final clean-up by adsorption chromatography. The PAC containing fraction was eluted with 25 ml DCM/Hexane (1:1, v/v), and reduced in volume by rotary evaporation and UHP nitrogen. The final extracts were stored under refrigerated conditions at 4°C in amber glass GC vials prior to instrumental analysis.

An Agilent 7890 gas chromatograph coupled with a triple quadrupole mass spectrometer fitted with electron ionization (EI) source was used for the acquisition of MS/MS spectra using an Agilent J&W HP-5 ms ultra inert column (30 m × 0.25 mm × 0.25 µm), with helium as the carrier gas at a constant flow rate of 1.2 mL/min. Sample (1 µL) was injected with a PAL RSI 85 autosampler at 250°C in splitless mode. Further GC/MS/MS and MRM conditions are outlined in Idowu et al. (2018) [22].

3.3.4. Data Analysis

Bioaccumulation Factors

Bioaccumulation factors such as biota-sediment accumulation factors (BSAF) are important parameters used when evaluating the partitioning behavior of PACs in the environment and subsequent risks to exposed biota. These BSAF values are also important when screening lower trophic invertebrates in environmental monitoring

programs as they can inform water quality at various sites over a study area. In our study, BSAF values were calculated using Equation 1

$$BSAF = \frac{Cb}{Cs} \quad (1)$$

Where Cb is the lipid normalized concentration of PACs (ng/g) in snails and Cs is the total organic carbon normalized concentration of PACs (ng/g) in surface sediments.

Nitrogen stable-isotope abundance relative to a standard (б15N) was calculated using Equation 2

$$б15\text{N} = \left[\left(\frac{\frac{15N}{14N\ sample}}{\frac{15N}{14N\ standard}}\right) - 1\right] x10^3 \quad (2)$$

In laboratory experiments, the enrichment in б15N in wildlife relative to their diet is on average +3.4‰ in a wide variety of animal taxa (Minigawa and Wada, 1986). The trophic level (TL) of each species was calculated based on the comparison of aquatic food chains using nitrogen isotopes in Cabana and Rasmussen (1996) [23] using Equation 3

$$TL = \left[\frac{(б15\text{N consummer} - б15\text{N reference})}{3.4}\right] + 2 \quad (3)$$

Where б15N consumer is the nitrogen stable-isotope enrichment value in wildlife consumer and б15N reference is the nitrogen stable-isotope enrichment value in snails [23].

The body residue of bioaccumulative and biomagnifying compounds in aquatic organisms often increases exponentially with increasing trophic level. The slope (m_1) of

the linear regression between log transformed tissue residues of a chemical (C_{LB}; lipid normalized values) and the organism's TL is also known as the trophic magnification factor (TMF), and is calculated using Equation 4 and Equation 5

$$Log_{10}(C_{LB}) = a_1 + m_1 \times TL \quad (4)$$

and

$$TMF_{m1} = 10^{m1} \quad (5)$$

When the slope (m_1) is significantly greater than zero ($TMF_{m1} > 1$), the chemical biomagnifies through the food web.

Statistical Analysis

Data were summarized and visualized using histograms and box plots of PAC residues in AOSR biota. A combination of analysis of variance (ANOVA) and analysis of covariance (ANCOVA) were used to determine if any statistically significant difference existed between BSAFs, the sum of different PAC compound types measured in different species (occupying different TLs) at different sites. Six different compound types were modeled, including parent lower molecular weight compounds; PLMW, alkylated lower molecular weight compounds; ALMW, parent higher molecular weight compounds; PHMW, alkylated higher molecular weight compounds; AHMW, parent heterocyclic aromatic compounds (i.e.- PACs containing N, S or O atoms); PHET, and alkylated heterocyclic aromatic compounds; AHET. Tukey's HSD post-hoc tests were used to determine which treatment combinations were significant. Statistical assumptions were verified using Levene's test and examinations of normal probability plots of residuals. When

assumptions were violated, non-parametric equivalents (ie- generalized linear models) were employed.

3.4. Results

A total of 94 biological samples were analyzed for PACs (including alkylated congeners and heterocyclic aromatic compounds; HETs), and stable isotopes (^{13}C and ^{15}N). Of these, 3 were pools of snails collected in the surface mineable area (SMA) at Saline Lake, in a steam-assisted gravity-drainage (SAGD) impacted area at Lac La Biche Lake and at Burnt Lakes, our low impact study area.

Eight lake whitefish, 10 northern pike, 13 walleye, and 60 river otters accounted for the remaining samples analyzed. Due to a zero-inflated dataset, and to help satisfy statistical assumptions, biota that had no detectable residues of any PACs were eliminated from the analysis. As a result, 13 river otters were removed from the dataset, with 81 samples remaining for statistical analysis (Table S3-1).

3.4.1. PAC levels in biota

51 different PAC compounds were analyzed in this study (Table 3-1). PACs had between 2-5 aromatic rings, with Log KOW ranging from 3.44 - 7.42 (Table 3-1). Method detection limits were all under 1.25 ng/g w.w. as reported in Idowu et al. (2018) [22]. Alkylated lower molecular weight compounds were the most common PACs detected (Table 3-2). Given the lipophilic nature of PACs, all PAC concentrations were normalized based on lipid content (i.e.- lipid weight normalization; l.w.). Wet weight standardized

data are presented in Table S1 for ease of comparison with other published literature. Number of samples collected are presented in Table S2.

PAC concentrations were highest in snails, ranging from 0 – 33,298.44 ng/g l.w. Higher trophic biota had PAC concentrations that were an order of magnitude lower or more (Table 3-2). For-example, river otter had alkylated lower molecular weight PACs ranging from 0 – 1,368.24 ng/g l.w. Parent lower molecular weight PACs, followed by alkylated higher molecular weight compounds were also commonly detected across species but at slightly lower concentrations and less frequently. Parent higher molecular PACs, and heterocyclic aromatic compounds (such as dibenzothiophene) and their alkylated congeners were detected at lower frequencies and lower concentrations. Snails were however the exception, having the highest concentrations and detection rates of all PAC compounds analyzed (Table 3-2).

Table 3-1 *Polycyclic aromatic compound (PAC) analysed in AOSR biota. PAC compound name, symbol, # rings, octanol-water partitioning coefficient (Log KOW), method detection limit (ng/g w.w.) and compound type are presented. PLMW = parent lower molecular weight, PHMW = parent higher molecular weight, ALMW = alkylated lower molecular weight, AHMW = alkylated higher molecular weight, PHET= parent heterocyclic aromatic compound, AHET= alkylated heterocyclic aromatic compound*

PAC	Symbol	# rings	LogKOW	MDL	Compound Type
Acenaphthene	At	3	3.88	0.67	PLMW
Acenaphthylene	Al	3	3.44	0.56	PLMW
Anthracene	A	3	4.55	0.43	PLMW
Benz[a]anthracene	Ba	4	5.74	0.59	PHMW
Benzo[a]pyrene	BaP	5	6.41	0.55	PHMW
Benzo[g,h,i]perylene	BghiP	6	6.89	0.33	PHMW
Chrysene+ Triphenylene	C + T	4	5.78	0.79	PHMW
Dibenzo[a,h]anthracene	DBahA	5	7.13	0.46	PHMW
Fluoranthene	Fa	4	5.19	0.52	PHMW
Fluorene	Fl	3	3.93	0.35	PLMW
Indeno[1,2,3-c,d]pyrene	IP	6	6.72	1.13	PHMW
Naphthalene	N	2	6.16	0.28	PLMW
Phenanthrene	Ph	3	4.58	0.76	PLMW
Pyrene	Py	4	5.13	0.45	PHMW
1,7-Dimethylphenanthrene	Ph1,7	3	5.34	0.30	ALMW
1,8-Dimethylphenanthrene	Ph1,8	3	5.34	0.30	ALMW
1-Methylnaphthalene	N1	2	3.78	0.57	ALMW
1-Methylphenanthrene	Ph1	3	5.04	0.32	ALMW
2,6-Dimethylphenanthrene	Ph2,6	3	5.34	0.30	ALMW
2-Methylnaphthalene	N2	2	3.79	0.57	ALMW
2-Methylphenanthrene	Ph2,6	3	5.04	0.33	ALMW
3,6-Dimethylphenanthrene	Ph3,6	3	5.34	0.30	ALMW
3-Methylphenanthrene	Ph3,6	3	5.04	0.33	ALMW
9/4-Methylphenanthrene	Ph9/4	3	5.04	0.30	ALMW
C1-Benz[a]anthracene/Chrysene	C1-Ba/C	4	6.18	0.33	AHMW
C2-Benz[a]anthracene/Chrysene	C2-Ba/C	4	6.59	0.79	AHMW
C2-Naphthalene	C2-N	2	4.24	0.51	ALMW
C2-Phenanthrene/Anthracene	C2-Ph/A	3	5.46	0.30	ALMW
C3-Benz[a]anthracene/Chrysene	C3-Ba/C	4	6.97	0.79	AHMW
C3-Naphthalene	C3-N	2	4.73	0.44	ALMW
C3-Phenanthrene/Anthracene	C3-Ph/A	3	5.90	0.76	ALMW
C4-Benz[a]anthracene/Chrysene	C4-Ba/C	4	7.42	0.79	AHMW
C4-Naphthalene	C4-N	2	5.22	0.39	ALMW
C4-Phenanthrene/Anthracene	C4-Ph/A	3	6.36	0.76	ALMW

Table 3-1 continued

Retene	Ret	3	6.12	0.76	ALMW
C1-Dibenzothiophenes	C1-DBT	3	4.86	0.43	AHET
C1-Fluorene	C1-Fl	3	4.37	0.35	ALMW
C1-Fluoranthene/Pyrene	C1-Fa/Py	4	5.26	0.26	AHMW
C2-Dibenzothiophenes	C2-DBT	3	5.33	0.43	AHET
C2-Fluorene	C2-Fl	3	4.82	0.35	ALMW
C2-Fluoranthene/Pyrene	C2-Fa/Py	4	5.56	0.45	AHMW
C3-Dibenzothiophenes	C3-DBT	3	5.81	0.43	AHET
C3-Fluorene	C3-Fl	3	5.32	0.35	ALMW
C3-Fluoranthene/Pyrene	C3-Fa/Py	4	6.38	0.45	AHMW
C4-Dibenzothiophenes	C4-DBT	3	6.29	0.43	AHET
C4-Fluoranthene/Pyrene	C4-Fa/Py	4	6.69	0.45	AHMW
Dibenzothiophene	DBT	3	4.34	0.43	PHET
Benzo[k]fluoranthene	BkF	5	6.4	0.44	PHMW
Benzo[b]fluoranthene	BbF	5	6.34	0.30	PHMW
C1-Benzo[a]pyrene	C1-BaP	5	6.84	0.55	AHMW
C2-Benzo[a]pyrene	C2-BaP	5	7.27	0.55	AHMW

Table 3-2 *PAC concentrations (ng/g lipid weight) measured in AOSR biota pooled across sites.*

	N	Mean	Median	Min	Max	Std.Dev.	Detection Rate
Snails							
Parent LMW	3	1612.99	154.32	69.17	4615.48	2600.58	100%
Alkylated LMW	3	11179.56	240.24	0.00	33298.44	19155.89	66%
Parent HMW	3	541.91	321.81	0.00	1303.92	679.25	66%
Alkylated HMW	3	6426.14	7296.63	0.00	11981.79	6038.14	66%
Parent HET	3	35.92	0.00	0.00	107.76	62.22	33%
Alkylated HET	3	11194.07	45.03	0.00	33537.17	19349.71	66%
Lake White Fish							
Parent LMW	8	6.20	0.00	0.00	38.58	13.33	38%
Alkylated LMW	8	33.45	24.89	0.00	79.01	25.87	88%
Parent HMW	8	5.91	0.00	0.00	47.27	16.71	13%
Alkylated HMW	8	43.90	7.84	0.00	252.85	86.46	50%
Parent HET	8	0.00	0.00	0.00	0.00	0.00	0%
Alkylated HET	8	0.33	0.00	0.00	2.68	0.95	13%
Northern Pike							
Parent LMW	10	45.94	11.89	0.00	372.75	115.16	70%
Alkylated LMW	10	553.35	133.49	0.00	4140.63	1268.79	90%
Parent HMW	10	0.00	0.00	0.00	0.00	0.00	0%
Alkylated HMW	10	184.50	82.41	0.00	785.24	261.12	70%
Parent HET	10	0.00	0.00	0.00	0.00	0.00	0%
Alkylated HET	10	14.71	0.00	0.00	86.81	31.64	20%
Walleye							
Parent LMW	13	169.64	5.83	0.00	1591.77	448.30	54%
Alkylated LMW	13	85.33	62.62	0.00	293.91	82.83	92%
Parent HMW	13	26.99	0.00	0.00	341.24	94.46	15%
Alkylated HMW	13	410.46	7.70	0.00	2598.98	833.83	54%
Parent HET	13	0.00	0.00	0.00	0.00	0.00	0%
Alkylated HET	13	27.56	0.00	0.00	133.02	42.69	46%
River Otter							
Parent LMW	47	26.42	0.00	0.00	185.03	42.34	49%
Alkylated LMW	47	131.23	51.86	0.00	1368.24	259.52	96%
Parent HMW	47	4.13	0.00	0.00	173.22	25.29	6%
Alkylated HMW	47	10.05	0.00	0.00	123.42	24.35	23%
Parent HET	47	0.00	0.00	0.00	0.00	0.00	0%
Alkylated HET	47	4.55	0.00	0.00	50.55	11.97	15%

Alkylated lower molecular weight phenanthrene/anthracenes as well as alkylated dibenzothiophenes were most frequently detected in PAC burdens of snails, especially in the SMA (Figure 3-2). Higher molecular weight benzo(a)anthracene/chrysenes accounted for most of the PACs detected in fish in downstream environments (i.e. SMA and PAD) with lower molecular naphthalenes being important contributors to PAC burdens in fish at lower impact sites (Burnt Lakes and SAGD). River otters most often had detectable residues of the same naphthalene and alkylated naphthalene compounds, especially at Burnt Lakes, but were substantially influenced more by highly alkylated phenanthrene/anthracenes as well as alkylated dibenzothiophenes, similar to snails, in the SMA and downstream in the PAD (Figure 3-2). Additionally, C3-Fluoranthene/Pyrene were measured at high concentrations in otters while they were not detected in any of the other biota analyzed in this study.

A one-way analysis of covariance (ANCOVA) was conducted to compare log 10-transformed sum of parent and alkylated PACs between fish species of different sex at the four different sites whilst controlling for age. Prior to modeling, the homogeneity of variance between groups was tested using the Levene's test and normality was assessed; all of the assumptions were met. The effect of age was not significant for both sum of parent ($F_{(1,22)}$= 1.88, p=0.18) and alkylated PACs ($F_{(1,22)}$=3.76, p=0.07). Sex was also not a significant predictor of the sum of the parent compounds and their alkylated congeners ($F_{(2,22)}$=0.98, p=0.39; $F_{(2,22)}$=0.14, p=0.87 respectively). Based on these results, and the fact that snails were not aged, and the age of river otters were evaluated categorically (older,

sexually mature breeding adults), both sex and age groups were pooled across all biota for further analyses.

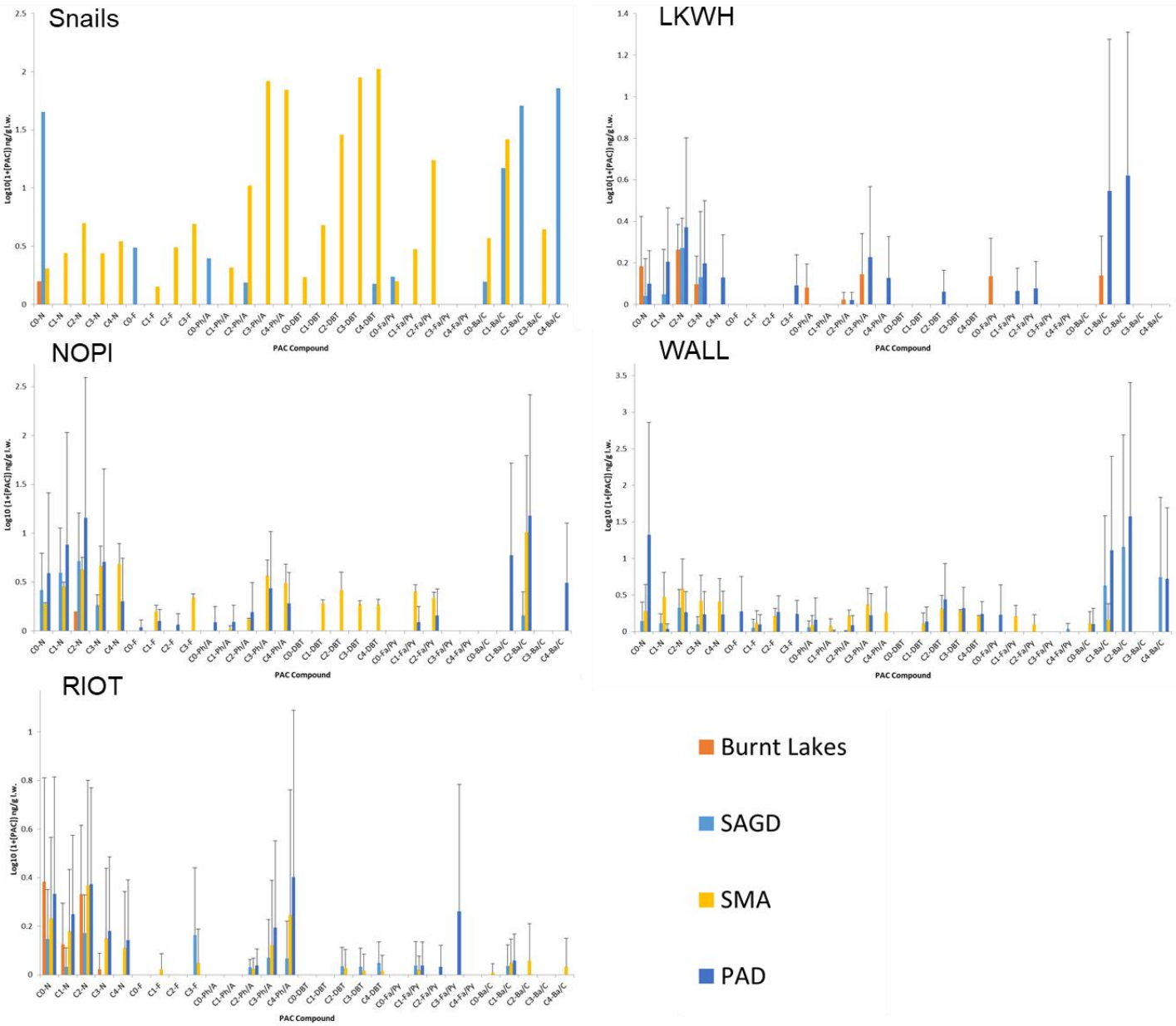

Figure 3-2 *Log transformed PAC profiles in AOSR biota, including snails, lake whitefish (LKWH), northern pike (NOPI), walleye (WALL) and river otter (RIOT). Levels are in ng/g l.w. and presented for PAC compounds of increasing molecular weight along the x-axis*

Log10 transformed sum of parent PAC and alkylated PAC were subjected to a main effect analysis of variance (ANOVA) with site (Burnt Lakes, SAGD, SMA, PAD) and species (snails, lake whitefish, northern pike, walleye, and river otter) entered as categorical predictors. The analysis revealed that the effect of species was significant for both parent, and alkylated PACs (Parent PAC: $F_{(4,73)}$= 4.01, p=0.005; Alkylated PAC: $F_{(4,73)}$= 8.75, p<0.001). The post-hoc Tukey's HSD test indicated that snails had significantly higher residues of parent PACs than any of the other species (p<0.01). The sum of alkylated PACs were significantly higher in snails when compared with river otter (p<0.01), and in northern pike when compared with river otter (p<0.04); all other species were not significantly different from each other. The ANOVA results also showed that the effect of site was significant, but only for alkylated PACs ($F_{(3,73)}$= 5.39, p=0.002). The Tukey's HSD post-hoc test indicated that alkylated PACs were highest downstream the oil sands in the PAD (p<0.003) followed by the SMA (p<0.04) when compared to Burnt Lakes, which is the lower impact study area (Figure 3-3). Care should be taken when evaluating the robustness of our inter-site differences in PAC concentrations across biota. We selected four different study areas in order to capture a range of PAC levels and sources in Boreal biota to investigate trophodynamics of PACs across sites. Species could unfortunately not be sampled equally between sites, and in some cases, some species were absent from a study area but present at another.

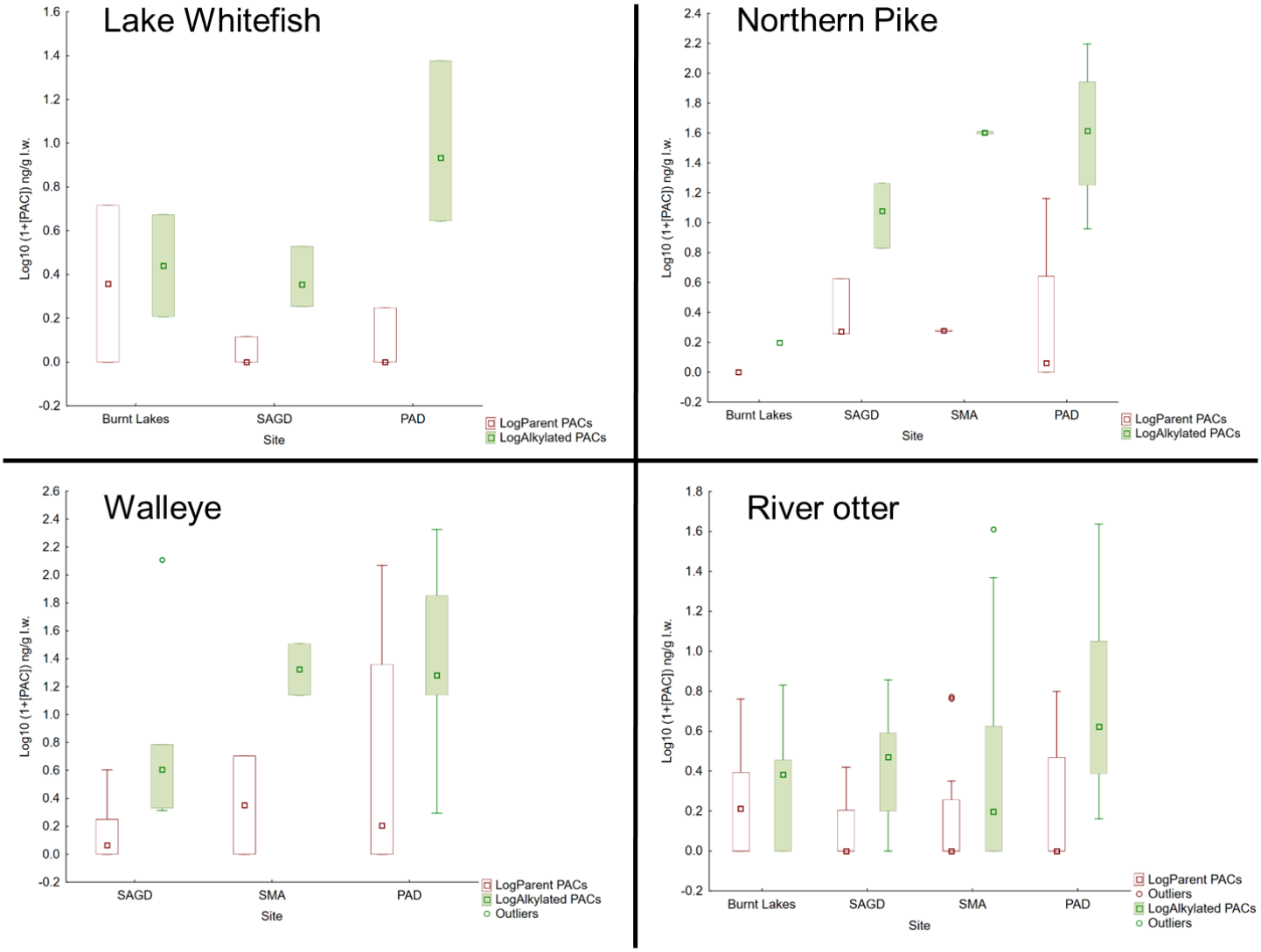

Figure 3-3 *Box plots of Log10 transformed sum of parent PACs (ng/g l.w. - red) and sum of alkylated PACs (ng/g l.w. - green) in Athabasca Oil Sands Region (AOSR) biota across 4 main study areas. Note that not all species were present at all four study areas. The center square of each box plot is the median, the box is the interquartile range (bottom is the first quartile, and top is the third quartile). The upper and lower whiskers represent the quartile 1st and quartile 3rd multiplied by the hinge value of 1.5.*

3.4.2. Bioaccumulation of PACs in Lower Trophic Aquatic Biota

The BSAF values of lipid normalized PACs as a function of total organic carbon (TOC) normalized sediments are presented in Table 3-3. Surface sediment (first 1.5 cm) PAC concentration values were obtained from a previous study (Thomas et al. 2020 – Chapter 2) for the same sites where biota samples were collected. No snails were analyzed downstream the SMA in the PAD. The average BSAF values for different PAC types (LMW, HMW, and HET) ranged from 0 – 1.30 (Table 3-3). Alkylated heterocyclic aromatics had the highest BSAF values in the SMA followed by parent lower molecular weight PACs in the SAGD site and alkylated lower molecular weight PACs in the SMA. This means that species exposures to PACs with a higher BSAF will accumulate contaminants at a higher rate. All other PAC types at our three study sites were not bioaccumulative (i.e. BSAF < 1.0).

A two-way ANOVA on log10 transformed BSAF values yielded a main effect for the site ($F_{(2,\ 123)}$= 21.17, $p<0.0001$). A Tukey's HSD test revealed that the average BSAF was significantly higher in the SMA (μ= 0.77, SD= 0.5, $p<0.001$), followed by SAGD (μ= 0.37, SD= 0.57, $p<0.001$) with Burnt Lakes having the lowest BSAF values (μ= 0.02, SD= 0.12, $p<0.0001$) on average. The main effect of compound type was non-significant ($F_{(5,\ 123)}$= 1.17, p=0.33). However, the interaction between site and compound type was significant ($F_{(10,\ 123)}$= 10.51, $p<0.0001$) indicating that the effect of compound type was greater in the SMA than at the other two lower impact sites (SAGD and Burnt Lakes).

Table 3-3 *Biota-Sediment Accumulation Factors (BSAF) of PLMW, ALMW, PHMW, AHMW, PHET and AHET PACs in snails collected in the AOSR. N is the number of PAC compounds behind calculations of mean, median, min, max, and standard deviation. No snails were analyzed from the Peace Athabasca Delta (PAD)*

	N	Mean	Median	Min	Max	Std.Dev.
Burnt Lakes						
Parent LMW	6	0.14	0.00	0.00	0.85	0.35
Alkylated LMW	20	0.00	0.00	0.00	0.00	0.00
Parent HMW	8	0.00	0.00	0.00	0.00	0.00
Alkylated HMW	8	0.00	0.00	0.00	0.00	0.00
Parent HET	1	0.00	0.00	0.00	0.00	-
Alkylated HET	4	0.00	0.00	0.00	0.00	0.00
SAGD						
Parent LMW	6	1.17	1.10	0.87	1.50	0.24
Alkylated LMW	20	0.08	0.00	0.00	1.67	0.37
Parent HMW	8	0.43	0.36	0.00	1.01	0.46
Alkylated HMW	8	0.54	0.00	0.00	0.75	0.75
Parent HET	1	0.00	0.00	0.00	0.00	-
Alkylated HET	4	0.26	0.00	0.00	1.05	0.52
SMA						
Parent LMW	6	0.14	0.00	0.00	0.85	0.35
Alkylated LMW	20	1.09	1.07	0.77	1.48	0.20
Parent HMW	8	0.46	0.62	0.00	0.91	0.39
Alkylated HMW	8	0.47	0.37	0.00	1.24	0.53
Parent HET	1	0.79	0.79	0.79	0.79	-
Alkylated HET	4	1.30	1.34	0.91	1.61	0.31

3.4.3. Biomagnification of PACs in Higher Trophic Aquatic Biota

Due to their low frequency of detection, parent higher molecular weight PACs (PHMW; detected in 9.8% samples), and parent heterocyclic aromatic compounds (PHET; namely, dibenzothiophene detected in 1.2% samples) were removed from the analysis. The 4 remaining PAC types were subjected to a one-way ANCOVA to determine if PAC concentrations varied across sampling sites, while controlling for trophic level.

Homogeneity of variance across sites was assessed using the Levene's test and normality of residuals evaluated through normal probability plots to confirm assumptions. The main effect of site was not a significant predictor of parent lower molecular weight compound residues detected in oil sands biota (PLMW; $F_{(3,76)}$= 0.21, p=0.89). As such, when controlling for trophic level, PLMW compounds did not differ between sites. The log10 transformed trophic level was a marginally significant predictor of PLMW residues ($F_{(1,76)}$= 3.95, p=0.05). The effect of site was not a significant predictor of alkylated lower molecular weight PACs (ALMW; $F_{(3,76)}$= 1.22, p=0.31) nor was trophic level ($F_{(1,76)}$= 1.46, p= 0.23). However, 29% of the variability ($F_{(4,76)}$= 8.99, p<0.0001, Adjusted R^2= 0.29) of alkylated higher molecular weight PACs (AHMW) in oil sands biota was explained by site and trophic level. In that model, there was a significant effect of site on AHMW levels in oil sands biota ($F_{(3,76)}$= 5.22, p= 0.003) and a significant effect of trophic level ($F_{(1,76)}$= 20.32, p<0.0001). Beta coefficients of log10 transformed AHMW levels in AOSR biota revealed that on average, Burnt Lakes AHMW levels were 0.4 times lower than those in the SMA. PAD AHMW levels were on average 0.41 times higher than AHMW levels in the SMA, while there was no significant difference in AHMW levels between SAGD and SMA sites. Finally, trophic level ($F_{(1,76)}$= 13.02, p= 0.0005) was also included in a model explaining 16% of the variability of alkylated heterocyclic aromatic compound (AHET) levels in oil sands biota ($F_{(4,76)}$= 4.91, p=0.001, Adjusted R^2= 0.16).

The slope of the relationship between PAC concentration and trophic level is also considered the trophic magnification factor (TMF). TMF is an important parameter that assesses the enrichment of pollutants across trophic levels (i.e. how lipid-normalized

concentrations of pollutants increase in biota up a food chain). Given the significance of trophic levels as predictors of AHMW and AHET PAC levels in the ANCOVA analysis, a linear regression was fitted to Log10 transformed AHMW and AHET levels as a function of trophic level in pooled biota (across sites). Samples were excluded from the analysis for a more representative relationship between PACs trophic position in those cases where no AHET or AHMW compounds were detected. The slope of this regression (Figure 3-4a) indicates there is a negative trophic magnification factor of AHMW PACs (b=-0.67, p=0.0004, R^2=0.35). AHET also had a negative trophic magnification factor indicated by the negative slope of the regression in Figure 3-4b (b=-0.57, p=0.01, R^2=0.33), which was slightly less than AHMW compounds (Figure 3-4a). Since these TMF values were all less than 1, there is no evidence that alkylated PACs biomagnified in a boreal food chain, in fact, we present evidence of the opposite, that there is trophic dilution of these compounds.

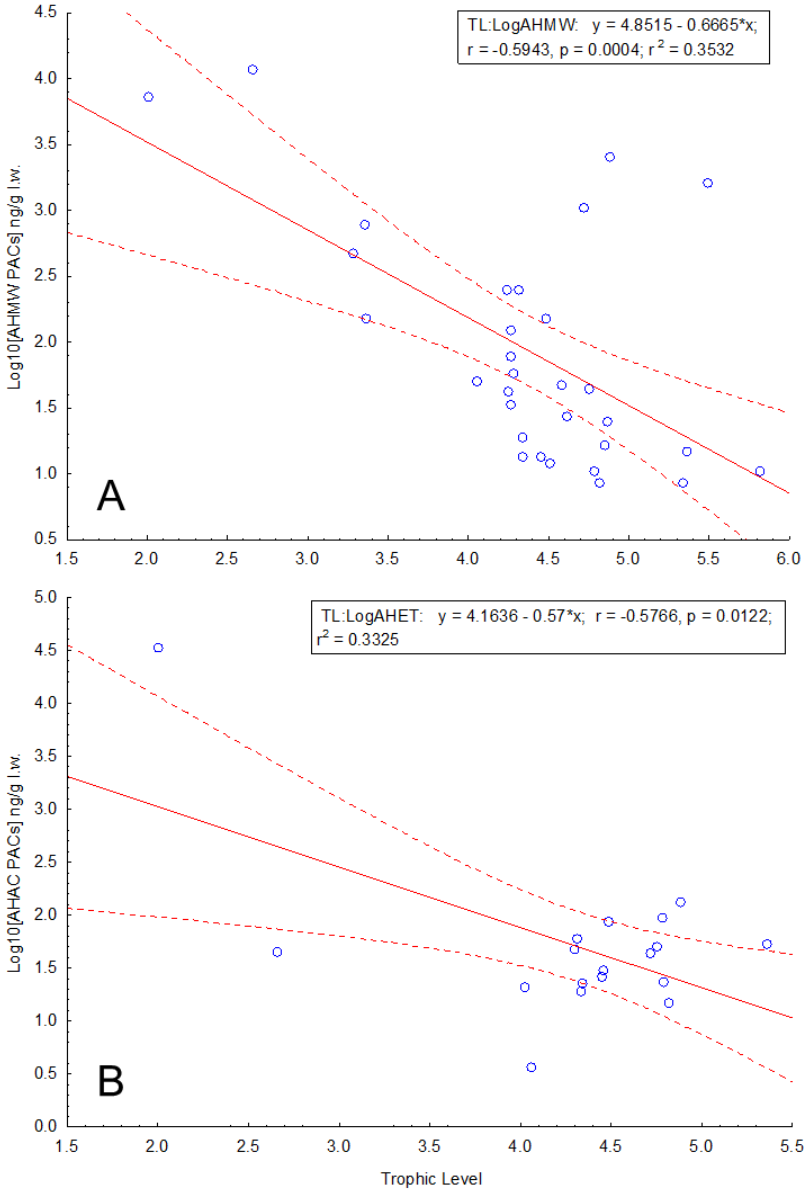

Figure 3-4 *Concentration of log10 transformed alkylated higher molecular weight (AHMW) PACs (A) and alkylated heterocyclic aromatic (AHET) PACs (B) as a function of trophic level. Slope of the relationship is the trophic magnification factor. Regression band and 95% confidence interval ellipses are presented.*

3.5. Discussion

3.5.1. PAC levels in AOSR biota

Samples collected in our study were dominated by alkylated PACs and had signature heterocyclic aromatic compound residue levels indicative of petrogenic sources. While there is often a debate on the bioaccumulation and biomagnification properties of PACs, our study provides evidence of higher bioaccumulation of PACs in lower trophic biota in PAC enriched environments with subsequent trophic dilution in higher order vertebrates. Few field studies measuring polycyclic aromatic compounds (PACs) in tissues of non-human biota from ambient concentrations in Canada have been published, and even fewer have investigated exposure to PACs and residue levels across food webs in the Boreal region of Canada.

While snails have never been sampled for PACs in the AOSR, snail PACs reported in our study fell inside the range reported in other lower trophic biota such as in various insect taxa in the oil sands [12] and wood frog (*Lithobates sylvaticus*) whole larvae collected in the AOSR [24]. Whitefish and walleye hepatic PAC residues were comparable to those reported in McMaster et al (2018) [19]. All other fish sampled fell inside the range of total PAC residues reported in the tissues of other fish species collected in the Athabasca River and its tributaries [25–28]. PAC residues have never been measured in the tissues of North American river otter (*Lontra canadensis*) living in the AOSR. Levels reported in river otter liver tissue in our study were comparable to those reported in sea otter (*Enhydra lutris*) whole blood collected on the central mainland of coastal British Columbia [16].

River otter hepatic parent PACs were higher than those reported in gull and tern eggs collected downstream of the AOSR in the Peace Athabasca Delta [29] but were lower than those reported in fecal samples of tree swallows, caribou, moose and wolf in the oil sands [30,31]. While these comparisons are helpful in evaluating the relevance of PAC burdens in AOSR biota, they should still be interpreted with caution. Comparisons between studies were difficult to make as they did not all consistently analyze the same suite of PACs and did not always report normalized PAC data the same way (i.e. wet weight vs dry weight vs lipid weight). Future studies should standardize how PACs data are presented in the literature. Given the fact that PACs are soluble in lipids [32], and that the lipid content of biota will influence PAC concentrations, future studies should report lipid normalized PAC data.

3.5.2. PACs in AOSR biota

While they were detected at low concentration in fewer samples, dibenzothiophene and its alkylated congeners were consistently detected in the SMA and downstream of the AOSR in the Peace Athabasca Delta (PAD) with concentrations being highest in lower trophic biota (i.e.- snails). Air monitoring studies including Schuster et al. (2015), Hsu et al. (2015) and Jariyasopit et al. (2016) consistently noted an abundance of alkylated-PACs (APACs) and dibenzothiophenes (DBTs and their alkylated congeners) over their parent compounds, with a corresponding decline in PAC concentration in air with distance from the SMA [33–35]. These distance-decay relationships were especially significant in APACs

and DBTs, even during forest fire periods, suggesting an association to petrogenic sources [34].

Similar to air monitoring studies, PACs in snowpack were dominated by DBTs, phenanthrenes/anthracenes (Ph/A), fluranthenes/pyrenes (Fa/Py), chrysenes (C), and fluorenes (F) [36–38]. PACs measured in snow were highly dominated by their alkylated congeners, especially near oil sands operations, and were attributed to particulates associated with mining activities [37,39]. Manzano et al (2017) further determined that sites <30 km from mining operations showed similar distribution of heterocyclic aromatic compounds to delayed petcoke (a carbon-rich solid material derived from the final cracking process in oil refining) [40]. A more concentrated spatial distribution pattern for these compounds suggested a more rapid atmospheric deposition process, implying a larger particle size, consistent with petcoke windborne fugitive particles [40]. DBTs, APACs and higher molecular weight PACs (such as C, Fa/Py) in biota were similar to trends published in air and snow, especially in the SMA and downstream to the PAD. Given their higher molecular weight and association to larger particle size deposition, exposure to these compounds in biota is most likely through ingestion (of snow and/or prey items).

At our lower impact sites at Burnt Lakes, and even at steam-assisted gravity drainage-impacted areas (incl. Lac La Biche Lake), lower molecular weight naphthalenes and their alkylated congeners were the predominant PACs detected in biota. Lower molecular weight (LMW) PACs (i.e. 2-3 ring compounds) tend to be more volatile. They are readily re-released to air or water even when bound to soils and sediments. By contrast, higher

molecular weight (HMW) PACs (i.e. 4, 5 or 6 ring compounds) are more tightly bound to sedimentary organic carbon so remain in these matrices for much longer periods [41]. The differing properties of low and high molecular weight PACs contributes to different exposures in free-ranging wildlife. For biota exposed to PACs at our low impact site (i.e. Burnt Lakes), a predominance of naphthalene and its alkylated congeners speaks to inhalation exposure pathways to ambient air PACs. While a predominance of HMW PACs in biota from the SMA or the PAD is a result of sediment ingestion (from direct contact with sediment, or from eating prey with sediments in their gut) as demonstrated above.

When monitoring PACs in biota, seasonal changes in PAC exposure are important to take into consideration. Seasonal trends in PACs were noted in some studies [33] with others even suggesting diurnal/nocturnal trends in PAC composition and distribution between gas-phase and particle-phase [42] that could result in effects on exposure and metabolism in free-ranging wildlife. For-example, the fact that C3-Fluoranthene/Pyrene were measured at high concentrations in otters while being absent in the other biota could be a result of the fact that otters were sampled during trapping season, in the winter, when these compounds have been shown to be prominent in snowpack in the SMA and downstream in the PAD [36–38]. The other biota were sampled in the summer, when lower molecular weight naphthalene and its alkylated congeners are found in water due to their greater water solubility and uptake by fish and other aquatic biota [26]. In 2019, fluoranthene and pyrene were added to the Candidate List of Substances of Very High Concern (SVHCs) by the European Chemicals Agency due to the compounds'

carcinogenic, toxic to reproduction, persistent, bioaccumulative and toxic (PBT) and very persistent and very bioaccumulative (vPvB) properties [43].

3.5.3. Trophodynamics of PACs

Biota-Sediment Accumulation Factors (BSAF)

Using top layers of sediment cores collected at the same sites as snails in SAGD, SMA and Burnt Lakes, it was possible to calculate a useful bioconcentration factor; specifically the biota-sediment accumulation factor (BSAF). BSAF values provide information on the partitioning behavior of PACs between sediment (the ultimate PACs sink) and lower trophic biota (like snails) that have direct interaction with sediment. In AOSR snails, BSAF values were highest for alkylated PAC compounds, and PACs with lower log Kow values (of lower molecular weight). This was especially true in the SMA with higher environmental PAC loadings, but not at lower impact sites such as Burnt Lakes and SAGD; most likely a reflection of the lower PAC concentrations detected in both sediment and biota at these sites. These results are supported by Wayland et al. (2008) that presented higher BSAF values for alkylated PACs vs their unsubstituted parent analogues. These results were attributed to alkylated PACs being more lipophilic and should, as a result, partition more in organismal lipids [12,44]. After an oil spill in Trecate, Italy, Brandt et al. (2002) measured PACs in soil, vegetation, insects, mice and frogs over space and time centered on the location of the blowout. Similar to our results, PACs with lower Kow coefficients and higher degrees of alkylation had higher BSAF values [45].

Generally, there is low bioaccumulation of chemicals with log Kow < 5, increasing to a peak bioaccumulation factor for chemicals with log Kow of about 7, and subsequently declining in chemicals with higher log Kow values [46]. It has further been suggested that the degree of lipophilicity is proportional to the degree of alkylation and the number of rings [47]. In our study, using values presented in McGrath and Di Torro (2009), average log Kow values of 5.4 were calculated for parent PACs while that average value increased to 5.6 for alkylated PACs suggesting that some bioaccumulation was possible [48].

However, bioaccumulation of PACs based on the interpretation of their Log Kow values did not explain the lack of a relationship in our study. Disregarding the effects of metabolism, and assuming that lipids and sedimentary organic carbon have the same affinity for PACs, BSAF values should be close to 1 according to the equilibrium partitioning theory [49]. While some alkylated PACs had max BSAF values approaching 2, median BSAF values for all PAC types in our study were reasonably consistent with a theoretical BSAF value of 1. This supports the idea that, overall, both parent and alkylated PACs partition relatively equally between organismal lipids and sedimentary organic carbon.

Trophic Magnification Factors

In Brandt et al. (2002), PAC biomagnification was not observed because PAC concentrations in lower trophic biota (including insects and mice) were lower than their diet items (i.e. plants). On the other hand, frogs had higher PAC residue levels (including PACs with high Kow coefficients) likely due to other exposure pathways (including dermal

absorption and sediment ingestion) [45]. In fact, trophic dilution of PACs was documented in a number of peer-reviewed journals, including a study by Fan et al. (2017) where the authors demonstrated trophic dilution in an aquatic food web of Lake Dianchi (China). In their study, the authors provided evidence that ingested PAC-containing sediment by benthos influenced trophic dilution rates [50]. In another Asian study, Wan et al. (2007) provided data supporting species-specific PAC profiles in Bohai Bay (North China) with subsequent trophic dilution in a marine food web with a length of four. The authors stipulated that their results were attributed to low PAC assimilation efficiencies and efficient metabolism at higher trophic levels [51]. In our study, apart from AHMW and AHET compounds, there were no significant relationships of PAC concentration with trophic level at any of the four sites in the AOSR. For the few significant compounds, trophic dilution was largely attributed to the efficient metabolic transformation in biota at higher trophic levels, since cytochromes P450 (part of mixed function oxidase systems that catalyze the oxidation of various chemicals) are relatively richer in higher trophic vertebrates [51].

However, assessing potential exposure to PACs by measuring tissue concentrations can be problematic due to high rates of PAC metabolism and excretion in vertebrates. When PACs are bioaccumulated, they induce metabolic pathways that stimulate transformation and elimination. The metabolism of PACs will change the nature and extent of exposure to PACs by changing excretion rates, the extent and duration of bioaccumulation (transfer from air, water, or food to tissues), and bioconcentration factors (BCF; ratio of PAC concentrations in tissues to concentrations in air, water, or food)

as was demonstrated in fish and birds [52–54]. In fact, Guomao et al. (2016) demonstrated through multiple linear regression analysis that biotransformation rates of PACs (incl. depuration rates) were more important factors compared with the lipophilicity of the chemicals (i.e. log Kow) in the assessment of trophic magnification potential of PACs in aquatic food webs [55]. In our study, given poor concordance of PAC Log Kow values with BSAF values, and negative TMF factors of two HMW PAC types (i.e. AHMW and AHET), ingestion, assimilation and subsequent depuration rates were more plausible explanations of trophic dilution relationships detected in AOSR biota.

3.6. Conclusion

In this study, we demonstrated how PACs exhibited trophic dilution in a Boreal food chain with river otters as apex predators. Bioaccumulation factors such as biota-sediment accumulation factors (BSAF) and trophic magnification factors (TMF) were used to evaluate the partitioning behavior of PACs in the environment and subsequent risks to biota. Our results revealed that alkylated PACs were highest downstream the oil sands in the PAD followed by the SMA when compared to Burnt Lakes, a lower impact study area. We also identified patterns of exposure to PACs in biota which were related to the different physico-chemical properties of the PACs. While we did not uncover any effect of age or sex in PAC residue levels across all biota, we did show that seasonality could be driving exposure to different PAC compounds. Further, PACs were determined to partition relatively equally between organismal lipids and sediment organic carbon suggesting that uptake mechanisms of PACs from sediment reaches equilibrium in lower

trophic biota. We determined that alkylated heterocyclic aromatic compounds and alkylated higher molecular weight compounds undergo trophic dilution in a Boreal food chain. Environmental risk assessments and environmental effects monitoring programs should especially focus on these compounds as their active metabolism could result in greater risks to exposed biota. Finally, our results suggest that ingestion, assimilation and subsequent depuration rates were more plausible explanations of trophic dilution relationships detected in AOSR biota than the lipophilic nature of PACs (ie- their log KOW). This suggests that BSAF and TMF values calculated in biota of the Northern Boreal region of Alberta, Canada might not be transferable to other sites. Environmental factors (including temperature and precipitation) could influence exposure, assimilation and depuration kinetics of PAC compounds in biota resulting in different trophic transfer relationships. Future studies should evaluate the spatial-temporal relationships of PAC exposure, assimilation and depuration kinetics in a representative food chain over a larger geographical area.

3.7. References

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Chapter 4.

Feces as a Biomonitoring Tool for River Otter (*Lontra canadensis*) population dynamics and hormonal responses in a landscape dominated by oil and gas anthropogenic disturbances

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4.1. Abstract

Polycyclic aromatic compounds (PACs) are a group of chemicals encompassing thousands of different aromatic, alkylaromatic and heterocyclic hydrocarbons. Several studies have highlighted increases in the concentrations of C1-C4 alkylated PACs and heterocyclic aromatic hydrocarbons such as dibenzothiophenes in the atmosphere, water, soil and sediments, plants and wildlife in the Athabasca Oil Sands Region (AOSR). There is increasing awareness that these chemicals may have profound endocrine disrupting properties in wildlife. Specifically, perturbations in steroid biosynthesis and signalling ultimately impair hormone homeostasis, which could have profound effects on reproduction at an individual and population level. North American river otter (*Lontra canadensis*) are good indicators of ecosystem health due to their ecology and sensitivity to environmental pollutants. River otters are also habitual users of latrine sites; providing a unique opportunity to collect non-invasive biological samples (feces) that can provide valuable information on a wide range of biological endpoints. In our study, river otter carcasses and matching fecal samples were used to establish dose-response relationships between hepatic PAC residue levels and fecal steroid hormone metabolites. Many PACs appeared to exert both positive and negative effects in male and female steroid hormone levels (including cortisol, progesterone and testosterone). While estradiol and T3 could not be modeled as a function of PACs, we showed how T3 was correlated to fecal steroid hormone metabolites. Three years of latrine site surveys and 105 total collected fecal samples at two trap lines in the AOSR managed to differentiate distinct fecal hormone metabolite responses in two different river otter populations under differential stress (ie

high (MOS) and low impact (SAGD) trap lines). Results of our population genetics analysis in field-collected scat samples revealed that river otters at the SAGD-impacted trap line had slightly higher genetic diversity as well as a higher effective population size consistent with higher fecal progesterone metabolite levels and a subsequent healthier population. While PACs are one of many stressors felt by free-ranging river otters living in oil and gas impacted landscapes, our results help in implementing sound conservation and mitigation measures.

4.2. Introduction

Canada's oil sands deposits are located primarily in the Peace River, Cold Lake, and Athabasca regions of northern Alberta [1]. Of these, the Athabasca Oil Sands Region (AOSR) is the largest and has undergone the most intensive development. The AOSR is situated wholly within Canada's boreal forest in the Mackenzie watershed, which is home to multiple Indigenous communities. Ecologically significant areas such as the Peace-Athabasca Delta (PAD; designated as a Ramsar Site under the RAMSAR Convention) and Wood Buffalo National Park (a UNESCO World Heritage Site) lie downstream of the AOSR [2]. Production in the Alberta oil sands has increased significantly, with recent forecasts estimating that the resource will contribute more than 1.7 trillion dollars to Canada's economy over the next decade [3]. However, despite the economic benefits of the oil and gas industry, there remain significant concerns regarding the impacts of increased oil production on the environment and human health; including the release and deposition of potentially harmful chemicals such as polycyclic aromatic compounds (PACs; [4–7]).

PACs are a group of chemicals encompassing thousands of different aromatic, alkylaromatic and heterocyclic hydrocarbons containing N, S, or O- atoms; 16 of which have been designated as priority pollutants by the United States Environmental Protection Agency (US EPA), the US Agency for Toxic Substances and Disease Registry (ATSDR), and the European Food Safety Authority (EFSA) due to their toxicity [8–10]. They are also listed on Schedule 1 of the Canadian Environmental Protection Act (1999). Several studies have highlighted increases in the concentrations of C1-C4 alkylated PACs and heterocyclic aromatic hydrocarbons such as dibenzothiophenes in the atmosphere,

water, soil and sediments, plants, wildlife and fish in the AOSR as a result of proximity to oil sands industrial activity [11–19]. Although there has been considerable research attention related to the toxic, carcinogenic and mutagenic properties of PACs, there is an increasing awareness that these chemicals may also have profound endocrine disrupting properties in wildlife [20–22]. Increasing evidence suggests that crude oil-related contaminants such as PACs and other heterocyclic aromatics may lead to altered thyroid hormone homeostasis, increased adiposity and impaired reproduction due to their endocrine-disrupting actions. [23]. For example, four ring PACs, as well as some of their alkylated derivatives and secondary metabolites, have been shown to exhibit strong anti-androgenic and estrogenic effects in vitro and in vivo through a variety of pathways including steroid receptor binding, alterations in steroid biosynthetic enzyme activity and metabolism of PACs to metabolites that can bind to steroid receptors (reviewed in [21,24–29]). Perturbations in steroid biosynthesis and signalling ultimately impair hormone homeostasis [27], which in turn can have profound effects on reproduction at an individual and population level.

The North American River Otter (*Lontra canadensis*) is found throughout North America in both freshwater and coastal marine environments. Otters and other semi-aquatic mustelids such as mink (*Neovison vison*) are considered good bioindicator species (i.e., a sentinel species) of aquatic ecosystem health due to their trophic position and their relative sensitivity to environmental stressors such as environmental contaminants [31–34]. As an apex mammalian predator, they may be susceptible to negative health consequences resulting from the bioaccumulation or biomagnification of persistent

environmental pollutants through their diets [33,35,36]. However, because of their elusive nature, monitoring river otters is challenging. Spatial-temporal demographic measurements like abundance, genetic variation, migration rates, and capture-mark-recapture (CMR) estimates are difficult to obtain for river otters [32,37,38]. Similarly, sex–specific demographic measurements are often lacking even under circumstances where different environmental stressors (such as exposure to endocrine-disrupting compounds; EDCs) may affect the demographic response of the sexes in different ways [39,40].

River otters are known for their habitual use of latrine sites. Latrine sites are areas that are scent-marked with feces, urine or anal gland secretions for either intra-group communication (marked more heavily in social groups) or mutual avoidance in nonsocial groups [41]. Latrine sites represent a unique opportunity for biomonitoring programs to study river otters using indirect sampling methods. Previously, the analysis of collected otter scat at these latrine sites has provided valuable information on a wide range of variables including reproductive steroid hormones [33,42], glucocorticoid metabolites to estimate physiological state [43], genomic DNA for individual identification [32,33,44], incidence of disease in wildlife [45], and dietary and food habits [41].

These latrine sites present a valuable opportunity to assess the impact of exposure to persistent organic pollutants (POPs) such as PACs on fecal steroid hormone levels and genetic diversity to evaluate individual and population health effects of PAC exposure in river otters. Measuring hepatic PAC residue levels in otters collected by trappers at the same sites can provide further information on recent exposures to environmental PACs.

By integrating these physiological measures (like fecal hormone metabolite levels and corresponding hepatic PAC residue levels) to population demographic estimates (like genetic diversity and effective population size estimates) obtained from fecal genomic DNA, population-level responses may be inferred to help us in determining population-level effects from exposure to PACs in oil-producing regions. Therefore, using an approach combining ecotoxicological and physiological analyses paired with population genetic estimates, our study investigated the following questions: 1) do otters exhibit dose-response relationships between hepatic PAC residue levels and steroid hormone metabolites measured in fecal samples collected from the rectum of carcasses; 2) do otters experience physiological compromises as a result of PAC exposures on two trap lines (each with multiple latrine sites for monitoring); and 3) do otter population demographics at the high impact trap line indicate a decreased population in response to PAC contaminant exposures; are these otters part of a sink population? We predict that otters with higher PAC contaminant exposures will respond through altered fecal metabolite hormone profiles with effects observed at the population level through less diversity in their fecal genetic profiles and smaller effective population sizes.

4.3. Materials and Methods

4.3.1. Study Area

All sampled trap lines were located in the Athabasca Oil Sands Region (AOSR), north of Edmonton (AB) and south of Fort McKay (just north of Fort McMurray, AB). This region also contains the largest oil sands deposit in Alberta, covering an area of about 46,000

km2 [46]. Latrine sites were surveyed at two trap lines; trap line A is just north of Fort McMurray in the Mineable Oil Sands region (MOS) and trap line B is in a steam-assisted gravity drainage-impacted area (SAGD) (Figure 4-1).

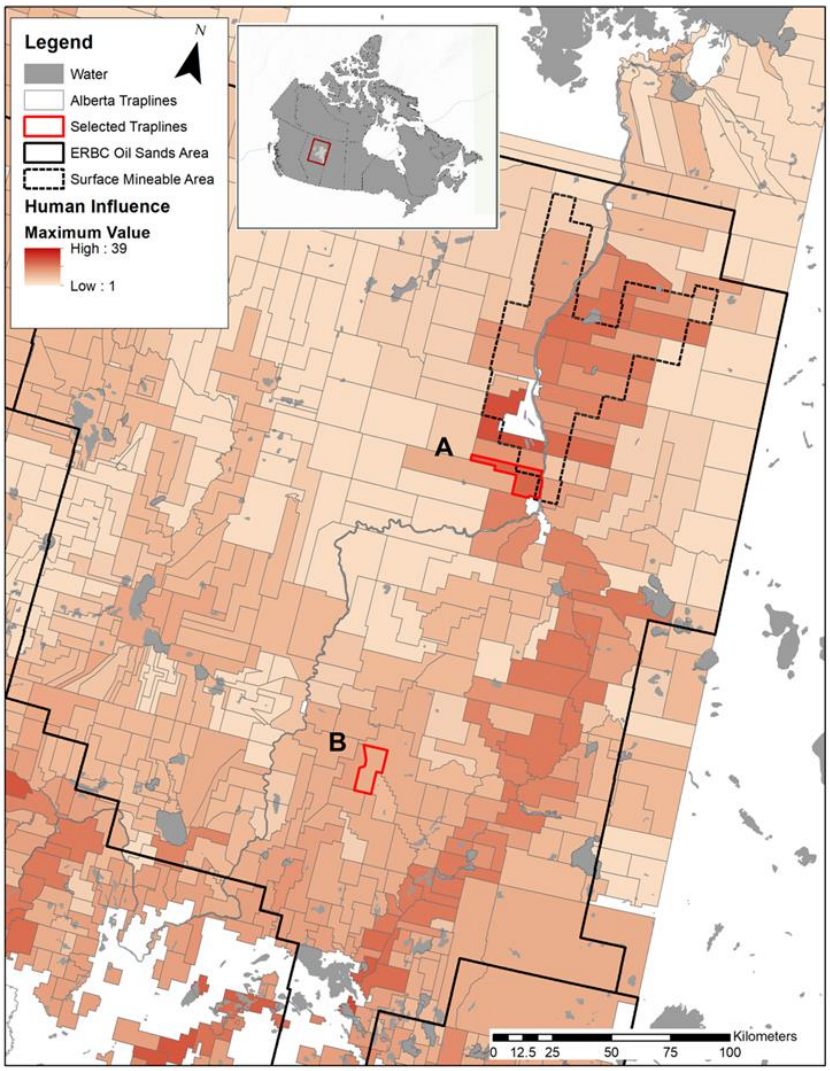

Figure 4-1 *Latrine sites were sampled at trap line A: Registered Fur Management Area #1790 (high impact; MOS) and trap line B: Registered Fur Management Area #1234 (low impact; steam assisted gravity drainage (SAGD)). The maximum anthropogenic disturbance per trapline is shown, where darker red indicates a higher anthropogenic disturbance. The location of the Oil Sands and surface mineable area is also presented.*

Assessing the relationship between exposures and responses in a complex ecological system can be difficult [47]. To address this issue, we used a geographic information system (GIS) and spatial information pertaining to anthropogenic disturbances on the landscape to support a before-after-control-impact (BACI) study design. The BACI study design is an effective method for evaluating how disturbances on a landscape affect ecological variables when treatment sites cannot be chosen at random [48], which is the case with the trapped river otters in our study. Implementing a spatially explicit framework allows us to identify patterns of disturbance across a landscape and use this information to appropriately select contrasting sites where dose-response relationships may exist [47,48].

In this paper we used an integrated spatial dataset that includes the location and magnitude of anthropogenic influence (i.e. population and dwelling density, roads, rail, utility corridors, mines, dams and reservoirs, etc.), which has a resolution of 250m and the ranked pixel values that range from 1 (no anthropogenic disturbance) to 51 (high anthropogenic disturbance) [49,50]. This layer was aggregated to the Alberta trap line layer to quantify the degree to which each trap line is impacted by human disturbance; we chose the maximum pixel value (highest rank of disturbance) as our summary metric. Based on this spatial framework, the MOS trap line (registered fur management area (RFMA) # 1790- trap line A) was considered as our high impact trap line while the SAGD trap line (RFMA # 1234- trap line B) was our low impact trap line; the maximum disturbance pixel value at the high impact site was 24 and the maximum disturbance at the low impact site was 13. The maximum impact of any trapline in Alberta was 39.

Additional information related to the historical PAC deposition and environmental loadings for neighboring sites and biota were outlined in Chapter 2 and Chapter 3 (paleotoxicological profiles of PACs in sediment cores and biota).

4.3.2. Wildlife Collections

To obtain river otters, Northern Alberta commercial trappers were recruited through the Alberta Trappers Association (Westlock, Alberta). River otter already trapped under permit for the commercial fur trade were collected from participating trappers. All animals were trapped following the Alberta Code for Responsible Trapping and the Agreement on International Humane Trapping Standards (AIHTS). Carcasses were frozen upon capture and then stored at -20°C until wildlife post-mortem evaluations, and dissections were conducted at the National Wildlife Research Centre (NWRC; Ottawa, ON). Gross necropsies were undertaken on each otter. Livers were dissected and stored in chemically-cleaned amber glass jars at -20°C for PAC analysis. Approximately 5g of feces were also collected from the rectum of otters, immediately before the anus, and stored in sterile Falcon tubes for dose-response hormonal analysis.

4.3.3. Field Sampling

Latrine sites were monitored at two trap lines during three subsequent winters (November and December 2013, 2014 and 2015) in Northern Alberta, Canada. Latrine sites were carefully selected to include areas that were shaded from the sun to avoid DNA breakdown. For 3 weeks, latrines sites were surveyed every day early in the morning, and new scat samples were swabbed for genomic DNA and collected whole for hormonal

analysis. Fecal samples were stored in 120 ml chemically-cleaned amber glass jars (Thermo Fisher Scientific, Ottawa, ON) and kept frozen at -20^{o}C.

Weather conditions included temperatures that ranged from +8°C to -24°C, light winds, and generally no precipitation. There were no systematic differences in weather conditions between both trap lines. At the high impact trap line in the MOS, 16, 14, and 7 latrine sites were monitored in 2013, 2014, and 2015, respectively. At the second trap line in SAGD impacted areas, 9, 11, and 12 latrine sites were monitored daily in 2013, 2014, and 2015 respectively. To ensure only fresh samples (i.e., < 24h old) were collected, once sampled, feces were covered with arts and crafts glitter [32], or pine needles to avoid swabbing the same sample several times.

After locating fresh fecal material, the outer mucus layer was swabbed with sterile cotton tip applicators (Thermo Fisher, Ottawa, ON) by gently running the cotton tip over a 3 cm2 area of the mucus layer. After rinsing the cotton tip in the DNA lysis buffer solution, a second swab was taken with the same cotton tip applicator. Samples were immediately placed in a microtube containing 1 ml of a modified DNA lysis buffer solution (4M urea, 0.2M NaCl, 0.5% N-lauroyl sarcosine, 10mM 1,2-ethylenediaminetetraacetic acid (EDTA), 0.1M Tris-HCl, pH 8.0; [51]). Samples in lysis buffer were stored at room temperature until DNA extraction.

4.3.4. PAC and Hormone Analysis

The analysis of PACs and their alkylated congeners in liver samples were undertaken at COGRAD, a Standard Council of Canada-accredited and ISO 17025 certified facility

following published methods in [52]. Briefly, approximately 5 g of liver homogenate was accurately weighed on a Sartorius Cubis MSE225P-100-DI (Fisher Scientific) and mixed with diatomaceous earth (DE). The mixture was loaded in an accelerated solvent extraction (ASE) cell, spiked with ^{13}C labelled internal standards and the dead volume of the cell filled with Ottawa sand. Procedural blanks, standard reference material (NIST SRM 2974a) and duplicates were included in each sample run. Samples were extracted under high pressure conditions with dichloromethane (DCM) used as the extraction solvent. ASE conditions are outlined in Idowu et al [52]. The extract volume was transferred to a rotary evaporator, evaporated down to 2.6 ml under ultra-high-purity (UHP) nitrogen, and mixed in with hexane (1:1 v/v) to a final volume of 5.2ml. 200ul of this volume was used to gravimetrically determine lipid composition. Removal of lipids from the extracts was achieved using automated gel permeation chromatography (GPC; J2-scientific AccuPrepMPS™, Columbia, Missouri, USA). The final extract was again reduced down to 1 ml under UHP nitrogen, and loaded on a silica/alumina column for final clean-up by adsorption chromatography. The PAC containing fraction was eluted with 25 ml DCM/Hexane (1:1, v/v), and reduced in volume by rotary evaporation and UHP nitrogen. The final extracts were stored under refrigerated conditions at 4°C in amber glass GC vials prior to instrumental analysis.

An Agilent 7890 gas chromatograph coupled with a triple quadrupole mass spectrometer (MS/MS) fitted with electron ionization (EI) source. Separations were performed on an Agilent J&W HP-5 ms ultra inert column (30 m × 0.25 mm × 0.25 µm), with helium as the carrier gas at a constant flow rate of 1.2 mL/min. Sample (1 µL) was

injected with a PAL RSI 85 autosampler at 250°C in splitless mode. Further, GC/MS/MS and MRM conditions are outlined in Idowu et al [52]. Polycyclic aromatic compounds analyzed included the same hydrocarbons as those listed in Table 3-1 (page 112).

Latrine (field samples; N=105) and fecal samples from carcasses (lab; N=36) were sent to the University of Guelph (Guelph, ON, Canada) for hormone metabolite measures. Testosterone, progesterone, corticosterone, triiodothyronine (T3) and estradiol were quantified in submitted scat samples. Fecal hormone extractions were carried out by first drying 1.0 g w.w. in an oven at 60o C for 24h. Following drying, samples were ground into a powder, diluted in 80% ethanol (0.2 g powder/5mL 80% Ethanol) in a glass vial and moved to a mechanical shaker. The vials were left on the shaker (1 pulse/second) at room temperature for 16 h, then centrifuged for 10 min at 500 x g. Supernatants were decanted and stored at 4^{o}C. Serial dilutions of fecal extracts were assayed with enzyme immunoassays (EIA) and gave displacement curves parallel to that of the respective standard curves [53]. Data for hormones are reported as ng/g dry weight fecal material.

Non-invasive fecal hormone metabolite evaluations are well established as a tool for monitoring physiological status in a variety of mammalian species [54]. Although the route of excretion of steroid hormone metabolites varies considerably among species, they still exhibit a similar pattern to those in plasma but with a lag time which can range from 12h to more than 2 days depending on the species [54]. For some hormones such as fecal estrogens, specific antibodies have been developed (or even antibodies against total estrogens) with little cross-reactivity. For others such as progesterone which is often

metabolized into several 5α- or 5β-reduced pregnanediones and hydroxylated pregnanes prior to excretion, relevant antibodies show considerable cross-reactivities with several pregnane metabolites [53]. As a result, care should be taken when specific progesterone antibodies are selected for the EIA [53]. In our case, we used the same antibodies as those previously published in Huang et al [33] and validated for *L. canadensis*. The detection limit for the ELISA assays were typically 0.28 ng/ml [55].

4.3.5. Population Genetics Analysis

Extracted DNA from the cotton tip applicators was used to amplify 11 microsatellites designed for North American river otters (Table S2; [44]) in single- and multiplex polymerase chain reactions. Additionally, a ~ 400-bp fragment of mitochondrial DNA (mtDNA) control region was amplified. All scat samples were amplified in a multi-tube approach (i.e., three amplifications) for all loci. Heterozygote genotypes were considered confirmed when the genotype was found twice, whereas homozygous genotypes were confirmed when the single allele was found three times [56]. The ConGenR script [56] was used to determine genotyping error rates. As allelic dropout is a major concern when genotyping fecal samples, we tested for a significant excess of homozygotes using the program Genepop v. 4.2 available online [57]. We also tested for potential genotyping errors, null alleles, and allelic dropouts using the software Micro-Checker v. 2.2.3 [58]. Finally, we compared generated microsatellite profiles in allelematch v. 2.5 [59] to identify matching genotype profiles. Refer to [44] for additional details on population genetics laboratory protocols.

Population genetic measurements like observed and expected heterozygosity, inbreeding coefficient, number of alleles as well as allelic richness were calculated with the programs GenAlEx 6.5 [60] and HP-Rare ver 1.1 [61,62]. Effective population size for each of the locations was estimated using NeESTIMATOR v2 [63]. Finally, an assessment of relatedness within each of the two sites (i.e., high and low impact trap lines) was obtained with the program ML-RELATE [64].

4.3.6. Data Analysis

Dose-Response Relationship

The relationship between hepatic PAC and fecal steroids was determined using matching liver and fecal samples collected from otter carcasses (N=34). Data were prepared for analysis by first recoding all PACs that were below the detection limit to zero. To avoid zero-biasing the models, all PACs with greater than 50% non-detect were removed from the analysis. To address the left-skewed distribution of the variables, a log10 transformation was chosen due to the broad range of measured PAC concentrations. All statistical analyses were completed in R 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria).

A Welch's two-sample t-test was used to determine if the measured fecal hormones varied significantly between sexes. While there was no effect of sex, all analyses still assessed males and females separately due to previously reported sex-specific differences in fecal hormone metabolite profiles in mammals [65,66].

Due to the large number of PACs measured, a principal component analysis (PCA) was performed to identify clusters of PAC variables before investigating its relationship with the measured hormones. A Pearson product-moment correlation between measured hormones in males and PAC concentrations and measured hormones in females and PAC concentrations were used to guide the forward step-wise regression model. Using an ordinary least squares regression (OLS), independent variables with the highest absolute correlation were sequentially entered into the model until variables were no longer significant. Non-linear models were also fit, but did not outperform the OLS regression models. For the purpose of these models, the α criteria of 0.05 was relaxed to include independent variables with $\alpha < 0.15$ [67]. Each fecal hormone dependent variable was modelled separately. Model fit was assessed using an Akaike Information Criterion corrected for small sample sizes (AICc) and was compared to the null model. The best model was assessed by standard regression diagnostics for residual normality, linearity, autocorrelation, and heteroscedasticity. Additionally, where there was more than one independent variable in the model, a variance inflation factor (VIF) was used to ensure there was no multicollinearity between the independent variables.

Latrine Site-Collected Fecal Samples

Fecal hormone metabolite level data in scat collected at the high impact (n= 27) and low impact (n=54) trap line were summarized and visualized using box plots. An independent-sample t-test was conducted to compare progesterone, testosterone, estradiol, T3 and cortisol levels between both high and low impact trap lines. Population

genetic analysis and demographic measures are summarized in data tables for interpretation.

4.4. Results

4.4.1. PAC Dose-Response Relationship in Fecal Hormone Metabolite Levels

Hepatic PAC Residue Levels

An independent-samples t-test was conducted to compare the log10 transformed concentration of parent PACs in males and females as well as the log10 transformed concentration of alkylated PACs in both sexes. No significant differences were detected for parent PACs in males ($\bar{x}$ (mean)= 0.92, standard deviation (SD)= 0.71) and females ($\bar{x}$=1.22, SD= 0.72; $t_{(34)}$= 1.24 p=0.22) nor were they detected for alkylated PACs in both sexes ($\bar{x}$=0.93, SD= 0.41; $\bar{x}$= 1.16, SD= 0.71 respectively; $t_{(34)}$= 1.2 p= 0.24). Total PACs ranged from 4.37 ng/g lipid weight (l.w.) to 121 ng/g l.w. in males while females ranged from 6.75 ng/g l.w. to 5,420 ng/g l.w. The individual with the highest concentration in the female dataset was a single young immature otter that had high levels of lower molecular weight PAC residues (Table 4-1). Specific PAC compound groups ranged from non-detectable lower molecular weight compounds in males to 3,390 ng/g l.w. in females (for the same parent lower molecular weight PACs; Figure 2). Except for lower molecular weight compounds, alkylated PACs were generally detected at higher concentrations (in higher molecular weight PACs and heterocyclic aromatics; Table 4-1, Figure 4-2). These data were used to model dose-response relationships as we had matching fecal samples collected from the rectum of the same otters.

Table 4-1 *PAC concentrations (ng/g lipid weight) measured in male (N=19) and female (N=17) river otters collected in the Athabasca Oil Sands Region (AOSR). PLMW = parent lower molecular weight, PHMW = parent higher molecular weight, ALMW = alkylted lower molecular weight, AHMW = alkylated higher molecular weight, PHET= parent heterocyclic aromatic compound, AHET= alkylated heterocyclic aromatic compound. High female values are due to a single outlier*

	Mean	Median	Min	Max	Std.Dev.
Males					
Parent LMW	18.20	5.17	0.00	90.10	27.40
Alkylated LMW	7.90	5.01	0.53	38.40	9.54
Parent HMW	2.36	0.54	0.00	13.00	3.85
Alkylated HMW	4.40	2.48	0.34	24.70	5.44
Parent HET	0.04	0.03	0.01	0.24	0.05
Alkylated HET	0.50	0.43	0.13	1.04	0.30
Females					
Parent LMW	216.00	8.85	2.83	3390.00	818.00
Alkylated LMW	104.00	5.51	1.52	1550.00	372.00
Parent HMW	17.40	0.51	0.00	262.00	63.30
Alkylated HMW	14.80	1.72	0.39	173.00	41.30
Parent HET	1.28	0.03	0.00	21.20	5.14
Alkylated HET	2.02	0.54	0.15	27.00	6.45

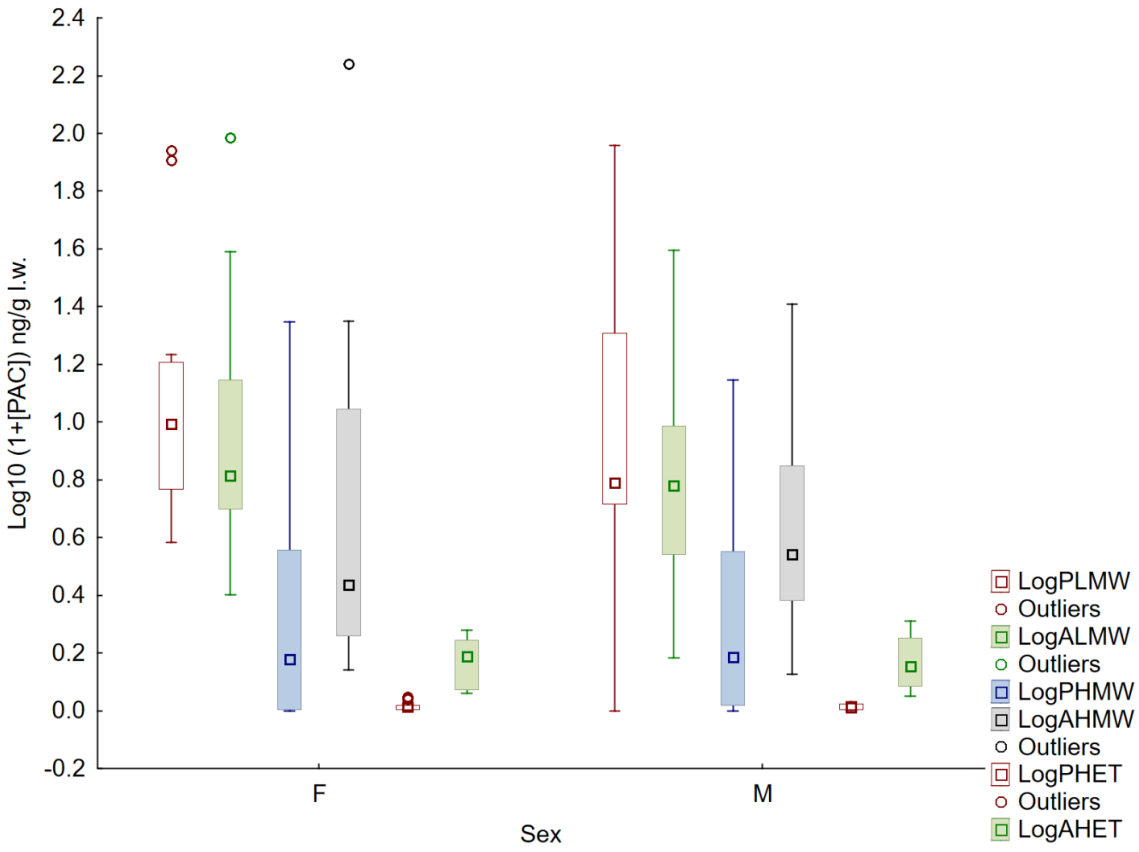

Figure 4-2 *Boxplots of Log10 transformed PLMW = parent lower molecular weight, PHMW = parent higher molecular weight, ALMW = alkylted lower molecular weight, AHMW = alkylated higher molecular weight, PHET= parent heterocyclic aromatic compound, AHET= alkylated heterocyclic aromatic compounds in male (M) and female (F) river otters (n=36) collected in the Athabasca Oil Sands Region (AOSR). The center dot of each boxplot is the median, and the box surrounding is the first quartile (bottom) and third quartile (top). The upper and lower whiskers represent the quartile 1st and quartile 3rd multiplied by the hinge value of 1.5.*

Fecal Hormone Metabolites and Dose-Response Relationships

First, a correlation matrix was explored to visualize the relationship between our 5 fecal steroid hormones to each other (Figure S2). T3 was most often correlated with the steroid hormones in both sexes. Testosterone in females also had significant relationships to some of the other hormones (Figure S2). T3 was most highly correlated with

progesterone in males and estradiol in females (Figure 4-3). However, a Welch's two-sample t-test revealed that there were no statistically significant differences in any of the steroid hormone levels (including the sex hormones) between males and females (Figure 4-4).

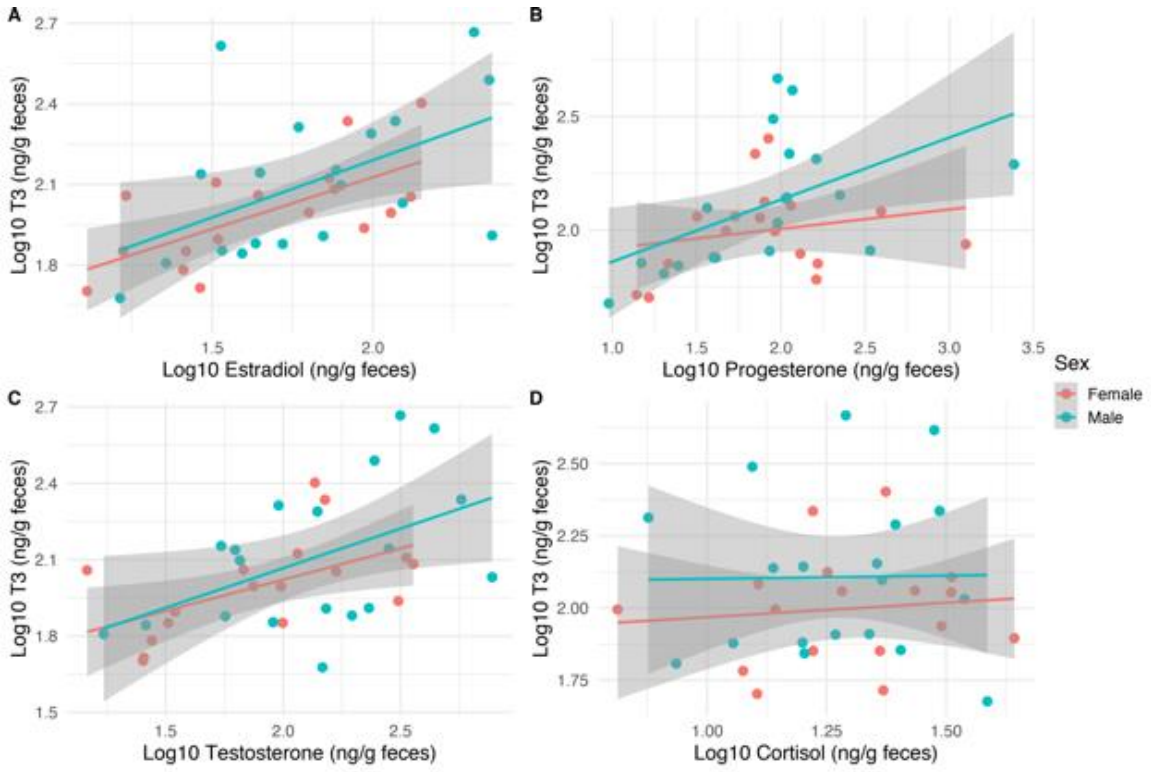

Figure 4-3 *Linear regressions of log10 transformed T3 concentrations in feces with steroid hormones in males (blue points and regression line), and females (red points and regression line). 95% confidence interval bands (light grey) are also presented.*

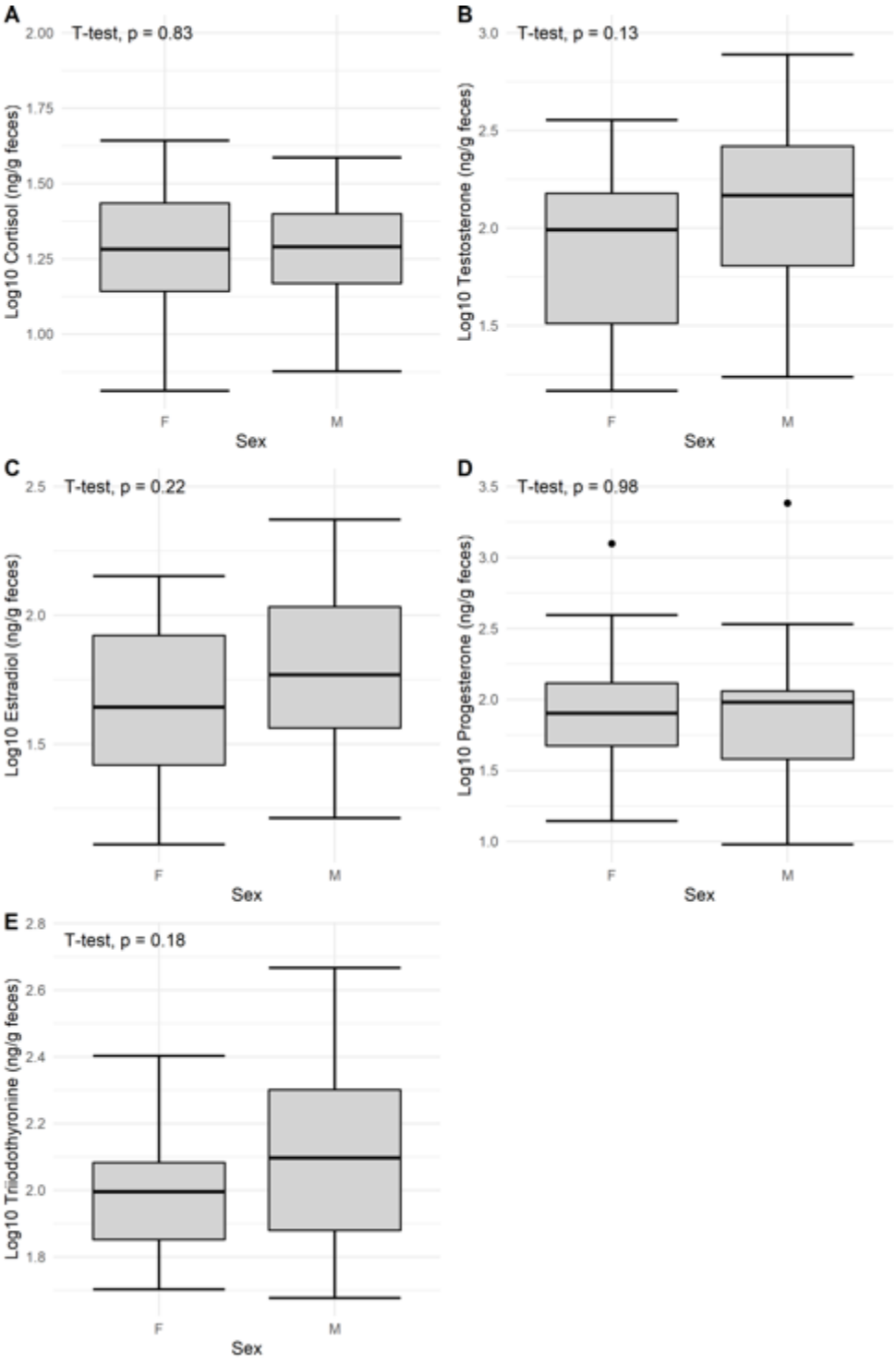

Figure 4-4 *Boxplots of the difference in fecal hormone metabolites by sex. The center line of each box plot is the median, the box is the interquartile range (bottom is the first quartile, and top is the third quartile). The upper and lower whiskers represent the quartile 1st and quartile 3rd multiplied by the hinge value of 1.5.*

A principal component analysis (PCA) was used as an exploratory data technique to assess which PACs clustered with the fecal hormones. Dim1 which explained 42.0% of variance in males and 73.8% of the variance in females (data not shown). The variance in Dim1 is driven by the skewed nature of the PAC data, and provided no useful information about the fecal steroid hormone levels. This was due to a zero-inflated dataset where even after Log10 and square root data transformations, normality could not be improved. This was made obvious when examining that PCA biplots that included Dim1 where the vector arrows (i.e.- loading scores) consistently clustered together. Dim2-Dim4 provided more useful information on which PACs can be related to the measured hormone concentrations. In both males and females, progesterone, testosterone and estradiol were correlated to each other (Figure S2). This relationship was also apparent in the PCA results where the same hormones clustered together in the same quadrants, indicating that they are similar and influenced together by the same compounds (Figure 4-5). In males, C3 Chrysene, C3-C4 Phenanthrene, Retene and C3 Chrysene have similar variance structure to the fecal steroid hormones. In females, C3 Chrysene, C2-C4 Phenanthrene, Retene, and C4 Naphthalene have similar variance structure to the fecal steroid hormones (Figure 4-5).

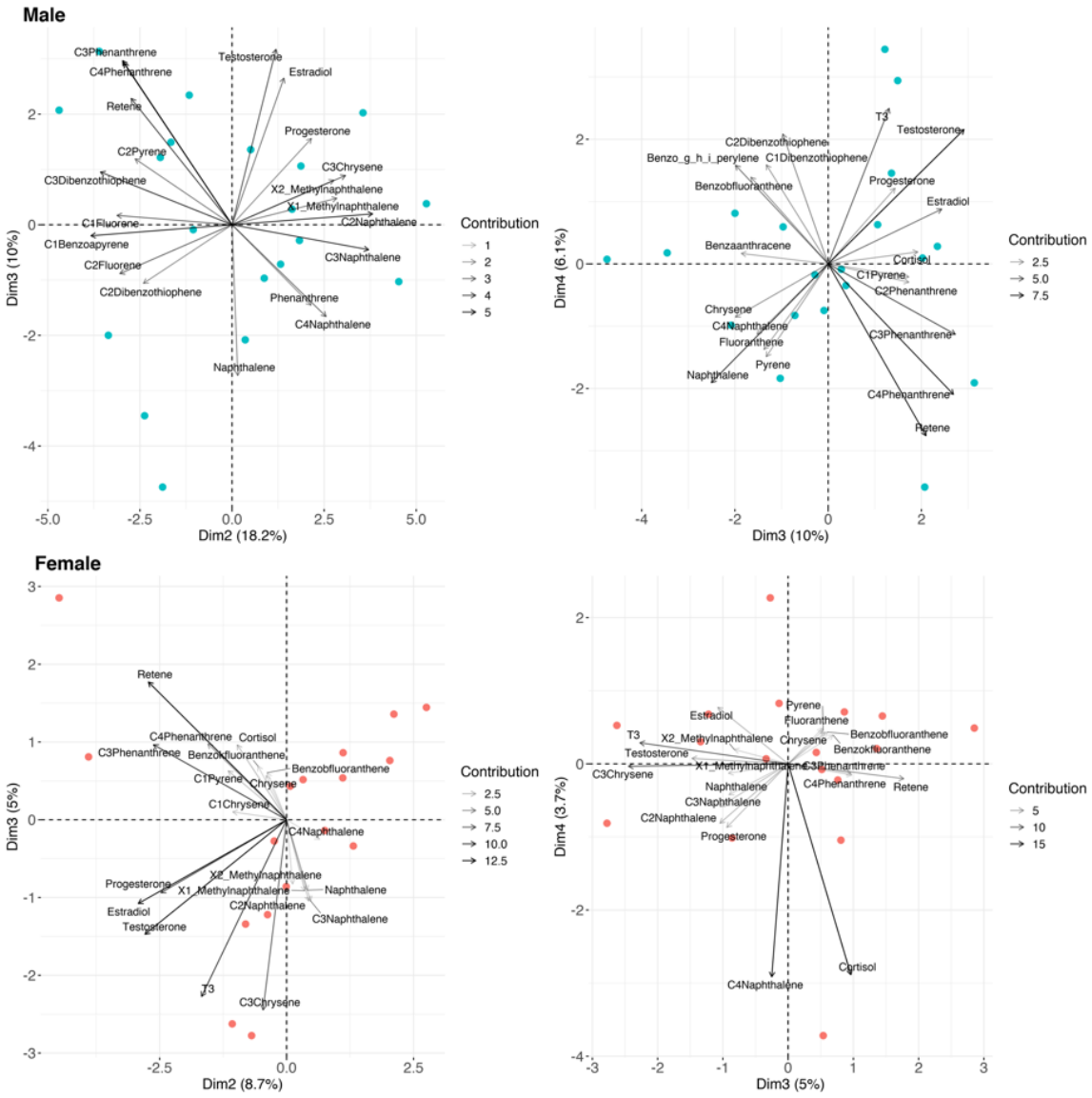

Figure 4-5 *Principal Component Analysis of steroid fecal hormone metabolites and PACs in males (top panes) and females (bottom panes). Dim2 – Dim4 are presented. The darker and longer the arrow, the greater that variable contributes to the variance structure of the PAC dataset.*

A forward stepwise model selection method was used to determine which of the PACs were good predictors of fecal steroid hormones. The analysis was run on the whole dataset, including the female otter outlier with high PAC hepatic residue levels. It should be acknowledged that this data point drove some of the relationships so care should be taken when interpreting these results. PACs had both positive and negative relationships with fecal steroid hormones (Table 4-2).

Table 4-2 *Summary results of forward stepwise regression model for each steroid hormone measured as a function of exposure to PACs in male and female river otter. Estradiol and T3 could not be modeled.*

Cortisol		**MALE**						**FEMALE**					
		Estimate	**Std. Error**	**T-value**	**P-value**				**Estimate**	**Std. Error**	**T-value**	**P-value**	
Intercept		1.1	0.09	12.6	<0.001			**Intercept**	1.18	0.06	19.49	<0.001	
C2-Phenanthrene		1.42	0.64	2.23	0.04			**C4-Naphthalene**	0.19	0.09	2	0.065	
								Retene	0.54	0.89	1.73	0.11	
Residual Std. Error	0.17	**DoF**	17	**R2**	0.23			**Residual Std. Error**	0.18	**DoF**	14	**R2**	0.31
ΔAICc	2.01	**F(1,17)**	4.96	**Adj R2**	0.18			**ΔAICc**	0.21	**F(2,14)**	3.28	**Adj R2**	0.12
		p-value	0.04							**p-value**	0.07		
Progesterone		**MALE**						**FEMALE**					
		Estimate	**Std. Error**	**T-value**	**P-value**				**Estimate**	**Std. Error**	**T-value**	**P-value**	
Intercept		1.83	0.13	14	<0.001			**Intercept**	1.8	0.13	14.28	<0.001	
1,8-dimethylphenanthrene		59.7	17.62	3.39	<0.001			**Retene**	6.13	2.29	2.67	0.02	
Pyrene		-1.44	0.61	-2.35	0.03			**Pyrene**	-0.54	0.25	-2.17	0.05	
Residual Std. Error	0.43	**DoF**	16	**R2**	0.38			**Residual Std. Error**	0.41	**DoF**	14	**R2**	0.37
ΔAICc	5.32	**F(2,17)**	6.6	**Adj R2**	0.008			**ΔAICc**	0.63	**F(2,14)**	4.12	**Adj R2**	0.28
		p-value	0.008							**p-value**	0.04		
Testosterone		**MALE**						**FEMALE**					
		Estimate	**Std. Error**	**T-value**	**P-value**				**Estimate**	**Std. Error**	**T-value**	**P-value**	
Intercept		1.87	0.13	14.25	<0.001			**Intercept**	1.97	0.1	19.44	<0.001	
1,7-dimethylphenanthrene		16.92	6.3	2.69	0.016			**Benzo(a)anthracene**	-0.6	0.3	-2.01	0.06	
Residual Std. Error	0.38	**DoF**	17	**R2**	0.3			**Residual Std. Error**	0.4	**DoF**	14	**R2**	0.21
ΔAICc	3.87	**F(1,17)**	7.22	**Adj R2**	0.26			**ΔAICc**	1.05	**F(1,15)**	4.03	**Adj R2**	0.16
		p-value	0.02							**p-value**	0.06		

Most PACs were positively correlated to fecal steroid hormone levels. Alkylated phenanthrenes (C2-phenanthrenes) had significant relationships with male cortisol, progesterone and testosterone (Table 4-2). Alkylated phenanthrenes (including retene, or 1-methyl-7-isopropyl phenanthrene) were also detected as being correlated to female progesterone levels. On the other hand, pyrene and fecal progesterone levels in both males and females were negatively correlated to each other (Table 4-2). Lower molecular weight alkylated naphthalenes were positively correlated to female cortisol while higher molecular weight benzo(a)anthracene had a negative relationship with measured testosterone levels in female otters (Table 4-2, Figure 4-6). Estradiol and T3 could unfortunately not be modeled.

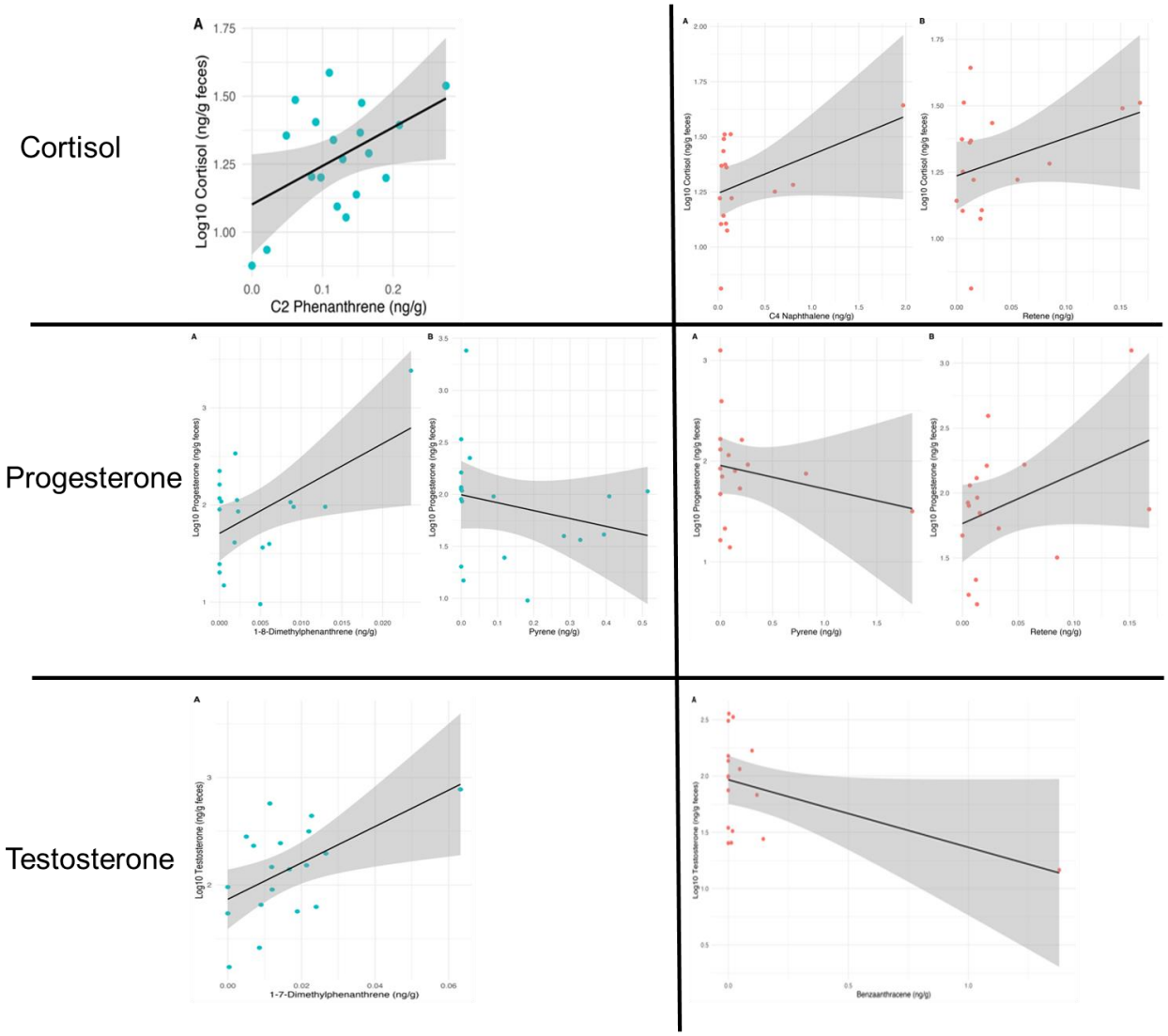

Figure 4-6 *Scatterplots of significant PACs on fecal cortisol, progesterone and testosterone in male (blue, left) and female (red, right) fecal samples. Partial linear regression and 95% confidence interval bands are presented. Estradiol and T3 could not be modeled as a function of PACs.*

4.4.2. Field Surveys

Hepatic PAC Residue Levels

River otters collected at the high impact (MOS) and low impact (SAGD) trap lines were analysed and described in Chapter 3 (bioaccumulation of PACs). Twenty five (25) river otters were collected from the mineable oil sands (MOS) region and 16 were collected from the in situ (SAGD) region (lower impact trap line). Parent PACs ranged from 0 – 233 ng/g l.w. while alkylated compounds ranged from 0 – 1,410 ng/g l.w. A main effects ANOVA revealed that there was no effect of site ($F_{(2,37)}$= 0.72, p=0.5) or sex ($F_{(2,37)}$= 0.22, p=0.8), meaning that both parent and alkylated PACs were not significantly different between MOS and SAGD river otters, and between males and females.

Latrine Site Surveys

Latrine survey sampling efforts for 2013, 2014 and 2015 are presented and discussed in Klutsch and Thomas (2018) [44]. A total of 105 fecal samples (including scat samples and matching DNA swabs) were collected. 31 samples were collected from the high impact trap line (MOS), and the remaining 74 samples were collected from the low impact trap line (SAGD). 81 samples had large enough quantities of scat for all 5 hormone enzyme immunoassays. 37% of these scat samples (30/81) could not be genetically sexed, leaving a single identified male at the high impact trap line. Given this unbalanced sampling design between sites and sexes, and the lack of any detectable differences between male and female fecal steroid hormones in an independent river otter dataset (section 4.4.1), both sexes were pooled (including individuals of unknown sex) in order to compare fecal

steroid hormones between MOS and SAGD trap lines with an optimal sample size (ie- n= 81).

Cortisol, T3, estradiol and testosterone were all significantly higher in river otter fecal samples collected in the mineable oil sands region (MOS; high impact) trap line (Figure 4-7). On the other hand, progesterone was higher in the scat of otters collected in the steam-assisted gravity drainage (SAGD; low impact) trap line. In fact, progesterone was not detectable in scat of otters in the mineable oil sands (Figure 4-7).

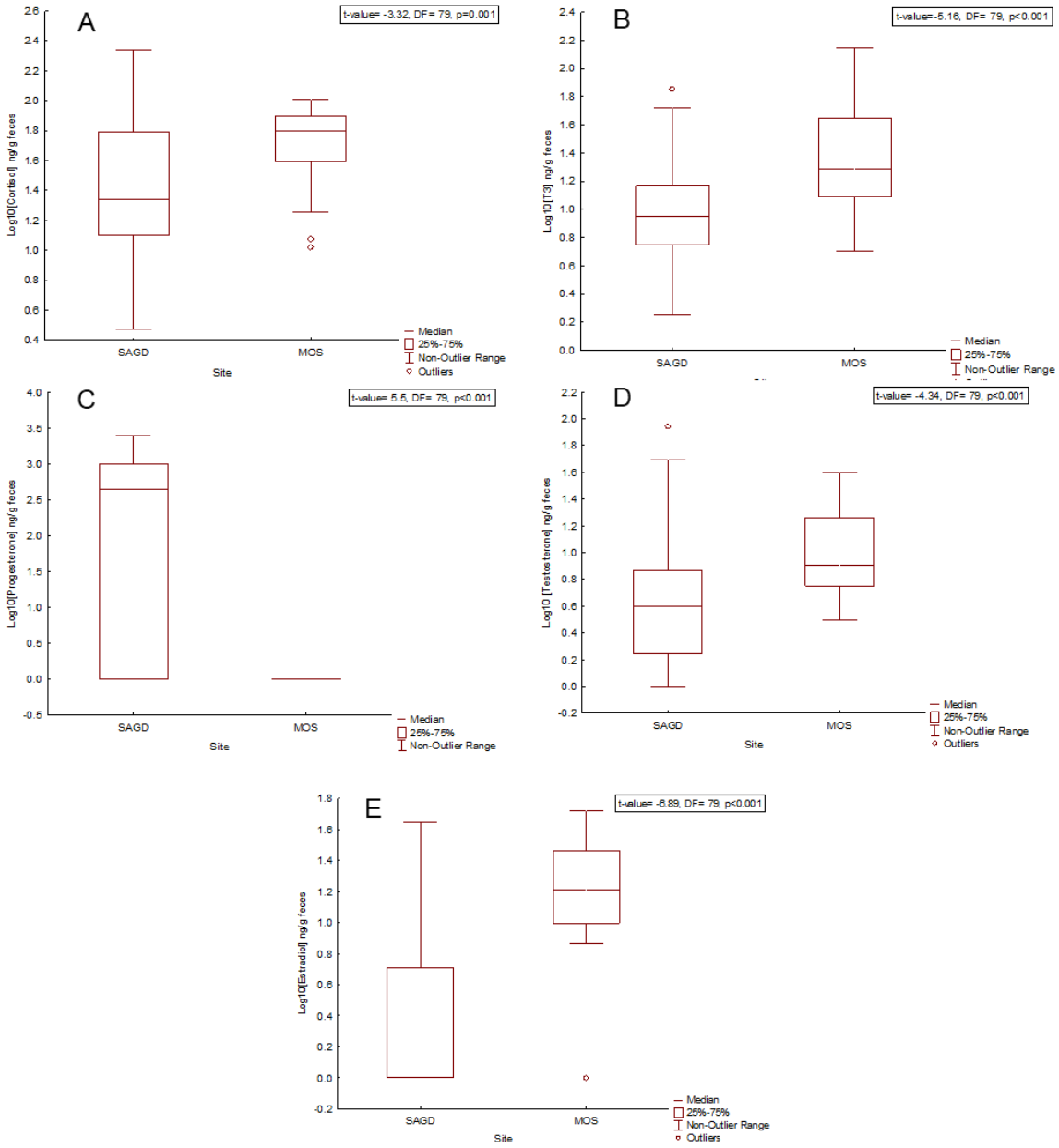

Figure 4-7 *Boxplots of log10 transformed concentrations of cortisol (A), T3 (B), progesterone (C), testosterone (D) and estradiol (E) in river otter scat samples collected on a SAGD trap line (low impact, N=27) and MOS trap line (high impact, N=54).*

4.4.3. River Otter Population Level Responses

A threshold of 11/14 loci was used to identify genetic samples of high quality. In total, 84/105 (80%) of our genetic samples met this threshold and were compared using the program Allelematch version 2.5 [59] to identify identical genotypes (ie- individuals). Of those 84 samples, 45 samples had unique genotypes and are therefore considered different river otter individuals. The 45 individuals included 13 samples from the MOS trap line and 32 from the SAGD trap line. Although sample sizes were adequate for statistical analysis, it should be noted that results for the MOS trap line are preliminary because of the still relatively small sample size (ie- 13 individuals identified). Genotypes of all 45 individuals were used in subsequent analyses.

We attempted to match fecal latrine samples to tissue samples collected from river otter carcasses submitted by trappers from both trap lines, at the same latrine sites. Eleven carcasses were submitted from the MOS trap line latrines, while 13 carcasses were submitted from the SAGD trap line latrines. Matches between fecal samples and tissue samples would provide a unique opportunity to temporally assess river otter physiological status as a function of hepatic PAC residue levels. Unfortunately, matches between latrine samples and carcasses were rare. In total, only three animals could be matched to latrine samples. While the sample size was too small for any meaningful statistical analysis, the low matching rate may suggest either that population sizes are very high and the sampling efforts do not capture all individuals or that there is a turnover of individuals.

The results of the population genetic analysis in latrine site-collected samples revealed similar genetic diversity estimates between both trap lines (Table 4-3). While the SAGD trap line seemed to have slightly higher genetic diversity, the differences were not significant (Figure 4-8). At the MOS trap line, effective population size was estimated to be 6.2 (95% CI: 2.9 – 10.7). At the SAGD trap line, effective population size was calculated as infinite, meaning that population size is very large using the coancestry method in NEESTIMATOR v2 [63]. Using an alternative method (i.e., linkage disequilibrium method), the general trend for effective population size could be confirmed with the MOS trap line having an estimated effective population size of 15.4 (95% CI: 9.4 – 30.2) and the SAGD trap line having an effective population size of 35.8 (95% CI: 23.0 – 67.1). Therefore, both methods indicated that the SAGD trap line had a higher effective population size.

Table 4-3 *Summary of genetic diversity estimates, averaged across 11 microsatellite loci for the two study sites (HIGH = highly impact site, LOW = lowly impact site). Number of samples (N), number of alleles (NA), allelic richness (AR) and private allelic richness (ARP) as calculated in HP-Rare [59,60], expected (HE) and observed heterozygosity (HO), FIS estimates, and standard errors (SE) for each of the estimates are given.*

SITE	N	N_A	SE	A_R	A_{RP}	H_O	SE	H_E	SE	F_{IS}	SE
MOS	13	4.786	0.689	4.63	0.85	0.618	0.049	0.606	0.051	-0.041	0.045
SAGD	32	5.214	0.656	4.57	0.79	0.617	0.041	0.641	0.043	0.028	0.030

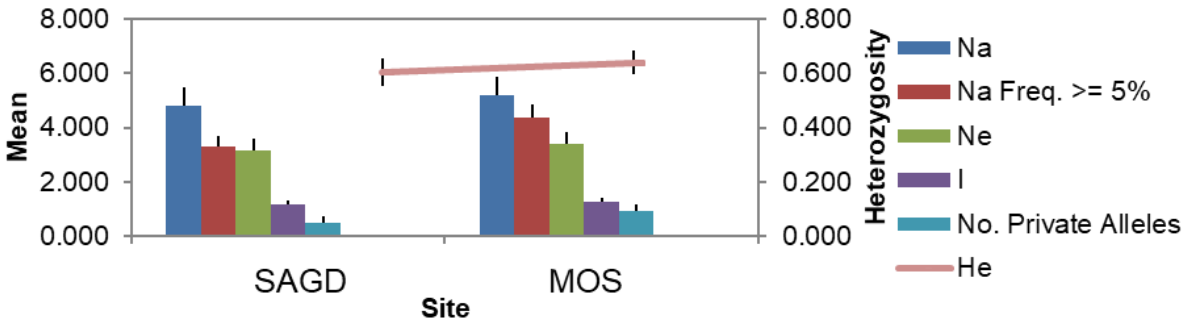

Figure 4-8 *Allelic patterns across the two sampling sites (SAGD = high impact trap line, MOS = low impact trap line). Number of alleles (Na), number of alleles with a frequency >= 5%, effective number of alleles, information index (I), number of private alleles, and trend for mean expected heterozygosity (He) are given.*

Relatedness estimates showed that there was a high number of unrelated individuals present at both trap lines. A matrix of relationships was used to categorize the maximum likelihood of relatedness into the following four relationship classes: unrelated (U), half-siblings (HS), full siblings (FS), and parent-offspring (PO). At the MOS trap line (high impact), only one parent-offspring pair could be found, whereas at the SAGD trap line (low impact), 12 parent-offspring pairs could be identified. Similarly, 55 half-siblings were found at the SAGD trap line, whereas only one half-sibling could be identified at the MOS trap line. There were 5 full siblings detected at the SAGD and 9 at the MOS trap lines.

Partial mitochondrial DNA (mtDNA) control region sequences (~400 bp) were obtained from fecal samples collected in the field, and in the lab during necropsies. Sequences were aligned and checked by eye using BioEdit [68] to ensure high data quality. Subsequently,

the program DnaSP v5 [69] was used to identify unique and matching haplotypes. The mtDNA amplification success rates were consistent with the microsatellite results and therefore, it was possible to produce mtDNA sequence data for almost all samples that amplified at microsatellite loci. Two previously identified haplotypes were identified in 2013 and 2014 (named H1 and H2). In 2015, a new, third haplotype named H5 could be identified. This additional mtDNA haplotype may indicate a recent immigration event to MOS trap line because it had not been found in previous years.

4.5. Discussion

4.5.1. Feces as a Biomonitoring Tool

Understanding the impacts of the natural resource extraction sector on the local environment is important for risk assessment purposes. However, monitoring wildlife responses to these stressors remains challenging at both the local and regional scale. Compounded by other confounding factors such as climate change and multiple other stressors, providing information on specific chemical stressor-response relationships in wildlife is made even more difficult. Reliable tools are needed to generate robust data useful to policy- and decision-makers.

Over the past decade, measures of adrenocortical activity, including measuring glucocorticoids (stress hormones) and glucocorticoid metabolites, have been used as an index of physiological stress experienced by wildlife [66,70-74]. Fecal hormone metabolite levels have been shown to provide an integrated measure of circulatory glucocorticoids. In turn, these are good representations of the physiological state of

target wildlife over time [75]. In fact, these measures are assumed to be more meaningful indicators over other alternative methods as they give a snapshot of longer-term physiological states and processes [76]. Consequently, depending on the research question, steroid metabolite concentrations measured in feces might represent the hormonal status of an animal more accurately than a single plasma sample, which only provides a snapshot in time of the physiological state [65]. For animals like river otter that are habitual users of latrine sites, feces are an important matrix for measuring steroid hormones (including both stress and sex hormones) to infer physiological status as these samples are readily available and easy to collect non-invasively without any animal handling [65,70,74,75].

In our study, fecal samples collected at latrine sites on two trap lines in the Athabasca Oil Sands Region showed different patterns in fecal hormone metabolites in two distinct river otter populations under differential stress (ie high and low impact) from the natural resource extraction sector. The differential stress in both populations could also be inferred from the cortisol results. Not only did we successfully measure fecal hormone metabolites, but the same fecal samples provided valuable genetic information that may be related to these anthropogenic stressors. These tools have demonstrable use to wildlife biomonitoring programs interested in the health of aquatic mammals.

4.5.2. Fecal Dose-Response Relationships

In chapter 2 (bioaccumulation of PACs in river otters), Thomas et al. (2020) provided evidence that environmental loadings of alkylated lower molecular weight

phenanthrene/anthracenes, as well as alkylated dibenzothiophenes, were high in lower trophic biota (ex. snails) in the MOS. As a result, exposure to PACs in higher trophic consumers should be higher in the MOS. Higher molecular weight benzo(a)anthracene/chrysenes were the main compounds detected in fish in the MOS and downstream receiving locations such as the Peace Athabasca Delta (PAD). On the other hand, lower molecular naphthalenes were important contributors to PAC burdens in fish at lower impact sites (including SAGD impacted areas). River otters most often had detectable residues of the same naphthalene and alkylated naphthalene compounds, but were more substantially influenced by highly alkylated phenanthrene/anthracenes as well as alkylated dibenzothiophenes, similar to snails in the MOS and downstream in the PAD. Alkylated Fluoranthene/Pyrene was also measured at high concentrations in otters while they were not detected in any of the other biota analyzed in that study. As a result, river otters physiologically responded to PACs present in their prey, or in lower trophic biota sharing the same environment.

Alkylated phenanthrenes (including retene) and alkylated naphthalenes were predictors of cortisol metabolites in the lab samples. The relationship was strong in males, but less clear for females as the relationship could have been driven by a few data points, notably one immature female otter with levels of PACs which were consistently more than 3x SD from the mean. Despite the slight discrepancies between sex, PACs were detected as having a positive association with fecal cortisol metabolite levels in an independent dataset of matching liver tissue with fecal samples collected from the rectum of otters in the lab. Taken together, these data suggest that the glucocorticoid

stress response in otters might be sensitive to anthropogenic perturbations in particular, to PAC exposure (amongst other stressors). Much of what is known on the impacts of exposure to PACs on glucocorticoid stress responses in aquatic wildlife focus on fish and most of these studies were field studies at polluted sites in temperate areas or laboratory-based studies on temperate fish species (reviewed in [77]). Very few studies have examined the impact of PACs and the stress axis in higher-order terrestrial vertebrates. Most studies agree that glucocorticoid stress responses in PAC exposed wildlife is a result of a number of factors, including pituitary atrophy leading to reduced adrenocorticotropic hormone (ACTH) release and subsequent cortisol secretion [78], the downregulation of key receptors (like MC2R) that mediate the response to ACTH release [79,80], and other impacts at multiple sites along the steroidogenic pathway leading to altered cortisol production [81]. Reductions in circulating cortisol levels is believed to be in part due to the activation of the aryl hydrocarbon receptor (AhR) leading to changes in the transcription of key genes including cytochrome P4501A1 (CYP1A) [82]. Cytochrome P450 family proteins play several key roles in the conversion of cholesterol to steroid hormones [81].

However, not all PACs are AhR agonists, and some studies have shown that exposure to PACs can also have stimulatory effects on the hypothalamic–pituitary–adrenal axis (HPA axis) and subsequent cortisol production and release [83–85]. Recent fish studies have shown that acute exposures to naphthalene, phenanthrene and complex PAC mixtures resulted in increased plasma cortisol levels [77,84,85] which could translate into higher fecal cortisol metabolite levels. Ringed seals experimentally exposed to oil

contaminants also exhibited increased plasma cortisol levels [86]. In a recent river otter study, non-POP related increases in fecal and blood cortisol levels were presented [33]. In their models, POPs (including OCPs/PCBs/PBDEs) were not significant predictors of fecal or plasma cortisol levels in wild river otter populations [33]. Inter-site differences in cortisol could be attributed to density-related issues and higher competitiveness at some sites [33]. In our study, PACs were significant predictors of fecal cortisol levels. River otters in the MOS had been previously shown as having higher hepatic residue levels of naphthalenes and phenanthrenes, the same compounds responsible for higher plasma cortisol levels in fish [77,84,85]. Fecal samples collected at latrine sites in the MOS also had higher fecal cortisol metabolite levels. Together, the weight of evidence suggests that PACs might play a role in altering cortisol biosynthesis in free-ranging river otters in the surface mineable area (MOS) of the oil sands.

Our study also identified significant relationships between PACs and progesterone levels. Progesterone levels were below detection limits in fecal samples collected at latrine sites in the MOS. The difference in progesterone profiles between sites, and the lack of any difference in between sexes was surprising. While pooled progesterone values across sites were comparable to basal fecal progesterone ranges in Bateman et al. (2009), the lack of any detectable progesterone in the MOS population could be a symptom of reproductive failure. Adult female *L. canadensis* are known to have distinct reproductive seasonality [87,88]. River otters are known to breed soon after parturition, in late winter/early spring followed by delayed implantation in females [88]. In mustelids such as river otter, a prolonged period of basal progesterone after ovulation corresponds to

embryonic diapause and can last between 7-9 months [87]. A subsequent increase in progesterone lasting between 68-73 days has been linked to embryo implantation [89]. As a result, monitoring river otter latrine sites for 3 weeks/year, always in late-November/early-December prior to estrus, was only a brief snapshot in the animal's reproductive cycle. Longer-term monitoring over longer temporal windows would give us more robust data useful to determine whether or not we detected actual reproductive failure. However, considering fecal samples at both trap lines were collected at the same time of year, the site-specific differences in river otter fecal progesterone were not anticipated and could be linked to exposure to contaminants such as PACs.

PAC exposure is known to inhibit ovarian steroidogenesis in flounder (*Platichthys flesus L.*) through inhibitory effects on cytochrome P450 17α Hydroxylase/17,20 Lyase (CYP17), 17B-HSD and P450-aromatase [90]. Exposure to environmental PACs has also been shown to affect ovarian follicle/granulosa cells, which could influence progesterone output in females [91]. While the exact mechanistic pathway of impacts to the biosynthetic enzymes and/or decreased aromatase activity remains to be determined in river otter, there is evidence that PACs could be impairing steroidogenic pathways in these semi-aquatic mustelids, starting with progesterone production. In support of this hypothesis, the average values of testosterone and estradiol in the AOSR fell orders of magnitude lower than those basal fecal metabolite levels in captive animals presented in Bateman et al. (2009). Similarly, when comparing T3, cortisol, testosterone, and progesterone fecal metabolite levels in wild North American river otters from coastal British Columbia [33], otters in the AOSR all had depressed T3, cortisol, and testosterone levels. However, to

confirm that these differences are related to PAC exposure will require the addition of more sites with a range of exposures as well as an unimpacted population in a similar environment (ie northern boreal forest). While the scope of this study did not include the determination of exact mechanistic pathways of effects from exposure to PACs, our results lend weight to steroidogenic impacts of PACs in free-ranging wildlife with possible mechanisms of action outlined in other studies.

4.5.3. River otter population demographics in the AOSR

The results of our population genetics analysis revealed that river otter populations at the SAGD-impacted trap line had slightly higher genetic diversity as well as higher effective population size. These results are consistent with published studies comparing contaminated versus reference sites [92–94] that showed lower genetic diversity and lower effective population sizes at contaminated sites. The relatedness analysis in combination with the effective population size estimates suggested that there was a higher number of breeding individuals and more closely related individuals at the low impact (ie- SAGD) site. This probably indicates that population health is higher at the low impact site than the MOS (high impact) site. In contrast, allelic richness and other population diversity estimates like observed and expected heterozygosity values were almost identical between populations. One possible explanation for this finding is that there is a high replacement rate at the MOS trap line leading to high diversity indices but lower relatedness and effective population size estimates. These results would also be consistent with impaired reproductive performance following exposure to EDCs in river

otters in the MOS. Thus, some diversity estimates may be misleading as monitoring tools and should be considered as part of a more holistic health assessment framework.

The results are also consistent with a source-sink dynamic model for the high impact site with higher loadings of environmental PACs. In this scenario, the high impact MOS site experiences relatively high immigration rates from adjacent areas that are contributing to high diversity indices but low relatedness values. This would be consistent with the clearly negative FIS value (Table 4-3) at the MOS trap line. Because of a smaller effective population size at the high impact trap line, it was expected that inbreeding would lead to positive FIS values; indicating mating of relatives. However, the opposite appears to be the case. Low numbers of new, unrelated individuals are migrating into the MOS area to breed, resulting in a negative FIS value indicating that mating is more random than expected by chance.

However, it is important to note that the sample size at the MOS trap line is considerably smaller than at the SAGD trap line, and therefore, it is recommended that future work focuses on increasing both spatial and temporal coverage of latrine site samples to corroborate our results. Increasing the scope of the sampling program would further allow us to determine whether the apparent outbreeding is caused by immigrants from nearby locations. If so, this would indicate a source-sink dynamic and a high population turnover in the MOS.

4.6. Conclusions

In our study, we managed to link PAC hepatic residues to fecal steroid metabolites in individual otters. These were then helpful in explaining fecal metabolite profiles in non-invasively collected fecal samples at latrine sites in the MOS (high impact trap line) and SAGD (low impact trap line) with known environmental PAC loadings. Population-level responses were then evaluated in light of these results. While PACs are one of many stressors felt by free-ranging river otters living in oil and gas impacted landscapes, our results can help in implementing sound conservation and mitigation measures. In order to implement sensitive conservation and management guidelines that also include monitoring the effects of human activities on an animal's environment/habitat in multi-stressor environments, long-term monitoring studies that adopt holistic wildlife health frameworks such as the one presented in this study are warranted.

4.7. References

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Chapter 5.

Conclusion

5.1. Summary of Main Results

Various studies have demonstrated the acute and reproductive toxicity of PACs to laboratory mammals [reviewed in 1,2]. Experimental studies in wild species have also demonstrated the potential toxic effects of PACs on other endpoints, some of which may be particularly important for free-living mammals, such as the suppression of immune function, altered food consumption and the presence of DNA adducts and associated increased carcinogenicity [3–6]. However, there are few published ecological studies investigating the chronic toxicity of PACs (including their alkylated congeners and heterocyclic aromatics) on wild mammal health and populations, and none in apex predators such as river otters in the Athabasca Oil Sands Region (AOSR).

There is evidence of increasing loadings of PACs via aerial deposition to aquatic environments in the AOSR [7–12]. This is evident from measurements of snow, and sediment cores of lakes up to 90km away from mining operations [7]. Increased loadings of PACs in these snow and sediment samples correlate to increases in mining activities since the 1960s, yet little is known on the biological impacts of exposure to PACs, and most importantly, their alkylated congeners and heterocyclic aromatic compounds in higher trophic vertebrates. As such, in this thesis, my goal was to provide critical information on environmental loadings of PACs in the AOSR, exposure and

bioaccumulation in Boreal biota at the same sites, and biological effects (i.e.- impaired endocrine responses and population level effects) in higher trophic vertebrates; the sentinel species, North American river otter (*Lontra canadensis*).

In Chapter 2, we demonstrated the utility of adopting paleotoxicological frameworks in defining environmental baseline levels of PACs and likely biological effects from exposure to these complex environmental mixtures. Using a paleotoxicological approach, this study combined analytical chemistry and a mammalian cell-based bioassay in dated lake sediment cores to assess historical toxicity of PACs in freshwater systems affected by emissions from the oil and gas sector and/or through natural forest fire and biomass combustion. Four sediment cores were collected from natural water bodies in the Athabasca Oil Sands Region (AOSR). Sediment intervals were radiometrically dated and subsequently analysed for PAC content and composition. PAC extracts from select dated intervals were used in cell-based bioassays to evaluate their endocrine disrupting properties. We demonstrated that there is spatial and temporal variability in the PAC composition of sediment cores around the AOSR with some of the highest levels of PACs and biological activity being detected near oil sands industrial activity. Our study is the first to link chemical analysis of sediment cores with endocrine function. This work demonstrates for the first time the feasibility of extending the usefulness of sediment cores in monitoring programs. While we observed no spatial or temporal differences in estrogen receptor alpha (ERα) mediated signaling, this approach demonstrates the powerful potential of coupling biological effects assessments to historical reconstructions of contaminant inputs to the natural environment.

In Chapter 3, we showed how PACs exhibited trophic dilution in a Boreal food chain dominated by river otters. Snails, prey and predator fish, as well as river otters were collected from four main study areas in the AOSR in northeastern Alberta, Canada. One site was at a downstream location in the Peace Athabasca Delta and Wood Buffalo National Park; a UNESCO World Heritage Site. The other three sites included areas marked by steam-assisted gravity drainage infrastructure (SAGD; centered on Lac La Biche Lake, Alberta, Canada); a natural wetland (Saline Lake) in the surface mineable area (SMA; Fort McMurray, Alberta, Canada) and a third site was 140 km west of the SMA in relatively pristine locations in the Birch Mountains (Burnt Lakes, Alberta, Canada). Bioaccumulation factors such as biota-sediment accumulation factors (BSAF) and trophic magnification factors (TMF) were used to evaluate the partitioning behavior of PACs in the environment and subsequent risks to biota. Our results revealed that alkylated PACs were highest downstream the oil sands in the PAD followed by the SMA, when compared to Burnt Lakes. Exposure to PACs in the SMA and downstream in the PAD are therefore more pronounced than at the lower impact sites. Lower molecular weight naphthalenes and their alkylated congeners were the predominant PACs detected in biota at our lower impact sites (incl. Burnt Lakes and SAGD). Lower molecular weight (LMW) PACs (ie- 2-3 ring compounds) tend to be more volatile than high molecular weight (HMW) PACs. LMW PACs in soils are readily released to air or water, and tend to be more bioavailable than HMW PACs. By contrast, higher molecular weight (HMW) PACs (ie- 4, 5 or 6 ring compounds) are more tightly bound to sedimentary organic carbon, so remain in these matrices and may be less bioavailable [13]. An animal's exposure to HMW compounds

may therefore result from ingestion of contaminated sediment, or inhalation of particulate rich in HMW compounds. As a result, our study identified patterns of exposure to PACs in biota that were related to the different physico-chemical properties of the PACs. While we did not uncover any effect of age or sex in PAC residue levels across all biota, we did show that seasonality could also be driving exposure to different PAC compounds in biota. Further, PACs were determined to partition relatively equally between organismal lipids and sediment organic carbon suggesting that uptake mechanisms of PACs from sediment reaches equilibrium in lower trophic biota. We determined that alkylated heterocyclic aromatic compounds and alkylated higher molecular weight compounds undergo trophic dilution in a Boreal food chain. These results suggest that ingestion, assimilation, and subsequent depuration rates were more plausible explanations of trophic dilution relationships detected in AOSR biota than the lipophilic nature of PACs (ie- their logKOW).

Finally, in Chapter 4, we successfully combined ecotoxicological and physiological analyses paired with population genetic estimates to investigate population-level responses to exposure to PACs. River otters are known for their habitual use of latrine sites. Latrine sites represent a unique opportunity for biomonitoring programs to study river otters using indirect sampling methods. Previously, the analysis of collected otter scat at these latrine sites has provided valuable information on a wide range of variables including reproductive steroid hormones [14,15], glucocorticoid metabolites to estimate physiological state [16], genomic DNA for individual identification [14,17,18], incidence of disease in wildlife [19], and dietary and food habits [20]. In our study, latrine sites were

used as a source of fecal samples for fecal steroid hormone metabolite analyses. First, river otter carcasses and matching fecal samples were used to establish dose-response relationships between hepatic PAC residue levels and fecal steroid hormone metabolites. We showed that most PACs exerted positive effects on fecal steroid hormone levels. Alkylated phenanthrenes (C2-phenanthrenes) influenced male cortisol, progesterone and testosterone, while alkylated phenanthrenes (including retene, or 1-methyl-7-isopropyl phenanthrene) also influenced female progesterone levels. On the other hand, pyrene had negative effects on fecal progesterone levels in both males and females. Lower molecular weight alkylated naphthalenes exerted a positive influence on female cortisol while higher molecular weight benzo(a)anthracene had negative consequences on measured testosterone in female otters. While estradiol and T3 could not be modeled as a function of PACs, we showed how T3 was correlated to the other steroid hormones. Three years of latrine site surveys and 105 fecal samples collected at latrine sites on two trap lines in the AOSR managed to differentiate fecal hormone metabolite profiles in two distinct river otter populations under differential stress (ie high (MOS) and low impact (SAGD) trap lines) from the natural resource extraction sector. Not only did we successfully measure fecal hormone metabolites, but the same fecal samples provided valuable genetic information used to reconstruct population-level responses to these anthropogenic stressors. The results of our population genetics analysis revealed that river otter populations at the SAGD-impacted trap line had slightly higher genetic diversity as well as a higher effective population sizes consistent with a healthier river otter population. These tools have demonstrable use to wildlife biomonitoring programs

interested in the health of aquatic mammals as they could help in implementing sound conservation and mitigation measures. In order to implement sensitive conservation and management guidelines that also include monitoring the effects of human activities on an animal's environment/habitat in multi-stressor environments, long-term monitoring studies that adopt holistic wildlife health frameworks such as the one presented in this chapter are warranted.

5.2. Research Contributions

5.2.1. Scientific Value

With millions of known chemicals in the world, some of which are frequently used in everyday life, releases to the environment are inevitable. Environmental pollutants can now be detected in almost every environmental compartment and can have serious consequences on ecosystem health. Organisms at every trophic level in various ecosystems are not immune to the risks from exposure to complex mixtures of anthropogenic pollutants; often exerting their effects through processes tied to reproduction and development [21,22]. Although some contaminants can degrade over time, others can be very stable and persistent in the environment. These persistent and inherently toxic contaminants are of particular concern to top trophic predators that are susceptible to exposure to high levels through bioaccumulation and biomagnification processes. As a result, these compounds are of importance to risk assessments due to their longevity and greater probability of occurring as complex mixtures in the

environment [23]. The exposure to complex mixtures of environmental pollutants adds a level of complexity when modelling risk of adverse health effect in wildlife.

The negative consequences of exposure to environmental pollutants can be mitigated through appropriate management strategies informed by sound environmental monitoring programs. Environmental monitoring under the Joint Canada-Alberta Oil Sands Monitoring (OSM) program was a response to stakeholder concerns regarding the environmental impacts of oil sands development in the region including the impacts of chemical pollutants on Indigenous traditional lifestyles. The OSM Program strives to monitor, evaluate, and report on the environmental impacts of oil sands development in northern Alberta, assess the risks of any impacts, and improve the characterization of the state of the environment. This includes evaluating the impacts of the industry's emissions on the environment including risks posed by increased exposure to complex mixtures of environmental pollutants. [24] The challenge remains as to how complex mixtures can be assessed as most of these chemicals are found in the environment with hundreds of thousands of other compounds. For example, PACs represent a single class of pollutants containing thousands of individual compounds. Furthermore, while many oil sands studies have clearly shown increases in environmental loadings of PACs, the biological interpretation of this increase is made more complicated by a lack of historical pre-development baseline data [25].

Recent studies have used paleolimnological approaches to address this knowledge gap and generate the much needed baseline environmental data [7–10]. We took this

approach one step further by showing how these samples can be used in combination with bioassays like Chemical Activated LUciferase gene eXpression (CALUX) assays to determine how historical PAC extracts can disrupt sensitive biological pathways such as endocrine processes. This tool becomes especially powerful when you consider the number of potential bioassays or omics technologies that could be used to inform mode of action or for the identification of bioactive compound(s) in the environmental extract. The bioassay-derived data are useful in contextualizing the potential biological relevance of changes in PAC deposition. These data can also fit inside effects-based assessment frameworks such as environmental effects monitoring programs (EEM) [26].

Introduced in the early 1990s in Canada, EEM programs are focused on the detection of biological effects rather than the detection of changes in chemical signatures [26]. EEM is an effects-based monitoring program that aligns itself with the concept of adaptive management; helpful when managing environmental changes linked to industrial development [27]. When designing EEM programs, challenges related to the setting of triggers (often after careful consideration of biological response values) are common. These triggers are used to enact change in monitoring intensity, focus, or research questions within EEM programs. Paleotoxicological frameworks such as the one presented in this thesis can help in defining change, setting trigger limits (based on, for-example, normal ranges of biological response values in sediment core extracts) and can help in identifying relevant biological pathways of effect.

Over the past decades, several strategies have evolved to overcome problems related to complex mixtures in screening processes and environmental monitoring programs and to provide tools capable of rapid and reliable substance detection and identification. Strategies integrating or combining separation sciences, mass spectrometry (MS), and biological screening allow us to screen complex chemical mixtures faster, and more reliably using a variety of procedures. In this thesis I show how these toxicological assessment tools may be applied in sediment cores to reconstruct the toxicity of past industrial emissions to retrospectively assess environmental impacts of past industrial procedures that may be used to regulate future industrial development.

However, assessing potential exposure to PACs by measuring tissue concentrations in biota and correlations with environmental loadings can be problematic due to high rates of PAC metabolism and excretion in vertebrates. When PACs bioaccumulate, they induce metabolic pathways that stimulate transformation and elimination. Transformation starts when some PACs bind to the aryl hydrocarbon receptor (AhR), resulting in the upregulation of mixed function oxygenase enzymes (MFOs) that constitute Phase I metabolism. The MFOs are primarily cytochrome P450 (CYP) enzymes CYP1A/1B [28], but a wide diversity of CYP isoforms (>450) exist in invertebrates [29] and vertebrates [30–32]. There can be marked differences in the induction of metabolic enzymes and biotransformation activity among taxa and species, and this is likely reflected in the PAC residue levels measured in Boreal biota (incl. snails, prey and predator fish, river otter) in the AOSR in this thesis. Some of these differences translate into differential toxicity of PACs among species [33] and the metabolism of PACs will change the nature and extent

of exposure to PACs by changing excretion rates, the extent and duration of bioaccumulation (transfer from air, water, sediment or food to tissues), and bioconcentration factors (BCF; ratio of PAC concentrations in tissues to concentrations in air, water, sediment, or food). Similar relationships among PAC structure, binding affinity to the AhR, and potency for inducing CYP1A enzymes have been observed in fish [34,35] and birds [36]. However, the relationships among the number of rings, ring conformation, and AhR binding affinity are confounded by parallel changes in molecular size (weight, dimensions) and KOW [37], all of which influence the uptake, transport, and disposition of PACs in tissues of aquatic organisms, and molecular interactions with receptors. For example, continuous exposures of fish to oil-containing sediments showed that accumulation of lower molecular weight (LMW) PACs reached a steady state relatively quickly, independent of PAC concentration, whereas higher molecular weight (HMW) PACs were taken up very slowly and did not reach equilibrium, even after four months of exposure [38]. In this thesis, poor concordance of PAC Log Kow values with bioaccumulation factors and negative trophic magnification factors (TMF) of two HMW PAC types (i.e.- alkylated PACs and alkylated heterocyclic aromatics) suggested that ingestion, assimilation and subsequent depuration rates were more plausible explanations of trophic dilution relationships detected in AOSR biota. As such, this thesis provides important data supporting the influence of PAC physico-chemical characteristics and metabolism on the levels of PACs and trophodynamics of PACs in different species.

Determining actual PAC exposures can be complicated as they are almost exclusively found in complex mixtures, consisting of multiple PACs, other contaminants, or photo-

transformed products. In addition, most Canadian field studies of PAC exposure have not measured biological effects within the same studies. However, measuring the body burden can help to clarify the link between exposure and effects as we did in the last data chapter of this thesis. In our study, we managed to link PAC hepatic residues to fecal steroid metabolites in individual otters. These data were then helpful in explaining fecal metabolite profiles in non-invasively collected fecal samples at latrine sites in the MOS (high impact trap line) and SAGD (low impact trap line) with known environmental PAC loadings. Population level responses were then evaluated in light of these results. While PACs are one of many stressors felt by free-ranging river otters living in oil and gas impacted landscapes, our results can help in informing sound conservation and mitigation measures and can also provide another layer of biological information helpful in setting trigger limits for EEM programs.

In this thesis, PACs were characterized and evaluated in sediment, lower and higher trophic biota with demonstrated impacts on endocrine processes and river otter population health. Effects-based assessments such as the one presented in this thesis are more powerful to environmental monitoring programs than stressor-based assessment methods (such as describing presence/absence or levels of contaminants) as they provide greater biological context to monitoring data, helpful in selecting triggers for EEM or adaptive management programs.

5.2.2. Social Value

Environmental and cultural impacts of oil sands activity observed by Indigenous communities in the AOSR are mostly constrained to Environmental Impact Assessments or Traditional Land-use Assessments to identify cultural land-use practices and significant sites to Indigenous communities in order to predict potential future impacts of industrial development [39,40]. Unfortunately, this type of 'knowledge extraction' risks knowledge transformation to a series of fixed coordinates (ex. Graves sites and trap lines) that are easily misrepresented, and do not capture the valuable, spiritual totality of the knowledge that shapes them [41]. Accountability in monitoring design permeates from the choice of research, method of collecting data, form of analysis and reporting of information that is meaningful and culturally relevant [40]. Over the past 10 years there have been many regional, multi-stakeholder groups that offered programs to support Indigenous communities, such as the Regional Aquatics Monitoring Program (RAMP), Cumulative Environmental Management Association (CEMA) and Lakeland Industry and Community Association (LICA). However, frustrations arose over processes and deliverables that were specific to the boundaries of the organizations that did not address key issues important to communities. The lack of trust in general monitoring practices became a result of inadequate community involvement and insufficiencies in integrating western science (WS) and Traditional Knowledge (TK) in monitoring designs. Not providing communities opportunity for meaningful collaboration forced many to abandon these initiatives to focus on indicators directly relevant to them. The use of culturally relevant wildlife health indicators in the context of environmental monitoring creates a point of connection that

shifts “existing externally imposed methodology…to a model that is culturally valid and meaningful to the communities impacted by environmental change” [42].

Indigenous people living in the AOSR practicing traditional rights and culture for centuries depend on healthy ecosystems for sustenance and maintenance of cultural lifestyles. Through years of lived experience on the land, TK holders have a unique ability to recognize change, drawing on the inextricable link between traditional practices and environmental health. This information is very useful when seeking baseline conditions across spatial and temporal dimensions, to identify and track environmental impacts in the AOSR that may not be recognized, or measured using only WS parameters.

The use of community-based monitoring (CBM) allows meaningful integration of TK through consistent interaction to better inform policy and decision-makers and build local capacity to collect and deliver ecological information from those experiencing it first-hand. CBM initiatives in the AOSR have filled research gaps through a ‘braiding’ of WS and TK. The integration of these knowledge systems enhances the relevance of research and monitoring programs in the AOSR by examining the relationships between chemical stressors and culturally relevant TK indicators (such as the health of river otter populations and other wildlife tied to water- like mink and muskrats). CBM can help guide WS in efforts to determine the what, where, and when in research and monitoring design. As an integrated approach, meaningful communication between collaborators involves sufficient training, resources and opportunities for knowledge sharing and Elder-youth exchange. CBM provides fundamental skills to empower communities, building capacity

to monitor the status and human health implications of consuming traditional resources. Together with WS, TK indicators such as change in colour, taste, abundance or physical abnormalities of culturally relevant species, can provide context to cumulative effects monitoring strategies, especially in locations or issues that may be under studied and potentially inform monitoring design.

The design and implementation of this thesis was done in collaboration, and after careful consultations with Treaty 8 Nations in the AOSR (incl. Lac La Biche Metis, Cold Lake First Nation, Red Earth Creek First Nation, Fort McMurray Metis, Fort McMurray First Nation, Fort McKay First Nation and Mikisew Cree First Nation, Athabasca Chipewyan First Nation and Metis Local 125 in Fort Chipewyan, Alberta). TK observations on changes in species abundance (including sensitive semi-aquatic furbearing species such as river otter and muskrats), reproduction and other traditional wildlife health indicators as well as observations tied to the abiotic environment helped in determining what to collect, where to collect it, and when to collect it. In return, capacity sharing exercises and knowledge exchange opportunities contributed to increased value for local communities.

The efforts undertaken in this thesis, including capacity provided by the University of Ottawa, supported the Strengthening Indigenous Employment in Environmental Monitoring Program; a training-to-employment and business development project that supports access of Indigenous peoples to local jobs in environmental monitoring, and contributes to strong ecological stewardship in their communities. Implementation funding (2016-19) was supported by the Strategic Partnerships Initiative, a federal

funding mechanism administered by Indigenous and Northern Affairs Canada. The program advances efforts to monitor the natural environment by linking to new and existing community-based monitoring initiatives, with a focus on water and wildlife health monitoring, while braiding Indigenous Knowledge and western science. Through the sharing of local Indigenous Traditional Knowledge and expertise, community-based monitoring programs can help inform monitoring program design, and can further assist in providing logistical support for the implementation of multi-year monitoring programs over large and remote landscapes; as they did for this thesis during sediment core collections, wildlife collections and latrine site surveys.

The methods developed and used for the research activities in this thesis contributed to CBM protocols delivered as part of an accredited training-to-employment program model that is rooted in Indigenous Traditional Knowledge, meets employers' needs, and opens doors to local employment opportunities with communities, governments, and industry in the domain of environmental monitoring. The collection of sediment cores, latrine site surveys, collection of DNA and wildlife dissections (and pathology) all contributed to a successful education program delivered on the land by an accredited institution.

5.3. Limitations and Future Work

The toxicity of PACs is most widely recognized as a product of the bioactivation of the parent compounds (through phase I and phase II metabolisms) into reactive oxygenated metabolites. As such, one must carefully select the bioassays for any paleotoxicological

evaluation. The living cells or tissues used in any bioassay should have the metabolic capacity to activate the PAC mixture into its more metabolically active secondary metabolites if any biological response is to be measured and modelled for risk assessment.

There can be marked differences in the induction of metabolic enzymes and biotransformation activity among taxa and species. Some of these differences translate into differential toxicity of PACs among species [33]. As a result, future work should focus on differentiating toxic responses to different environments with varying levels and different PACs. The “what to monitor”, “where to monitor” and “when to monitor” should continue being defined in close consultation with traditional land users and Indigenous communities.

Any field monitoring program is susceptible to the impacts of unbalanced designs due to poor sample sizes. We are never sure how many biota samples can be collected, how many latrine sites can be found, or how many fecal samples can be analyzed. In our last data chapter, sample sizes in the MOS could have been improved to reduce variability and increase our power of detection. By relying on local partners, traditional land users, and Indigenous CBM programs, more ground could be covered, and sampling would be more efficient. As a result, in continued collaboration with indigenous CBM, future work should focus on increasing both spatial and temporal coverage of latrine site samples and biota collections to corroborate our results. Increasing the scope of the sampling program would further allow us to sample otters from outside populations to evaluate PAC

exposure, physiological status and population dynamics to inform sound decision making for large monitoring programs.

Through the work undertaken in this thesis, I demonstrated the utility of using paleotoxicological frameworks in documenting historically-relevant biological changes in sediment cores. These data are helpful in evaluating historical changes in not only PAC deposition, but also likely biological effects from exposure to these contaminants in aquatic ecosystems. I then used this historical baseline to evaluate the trophodynamics of PACs in a Boreal food chain at the same sites, with river otters as apex predators. While there were site differences in PAC composition and concentration in biota, PACs were generally observed to undergo trophic dilution most likely due to increased metabolic capacity in higher order vertebrates. Finally, I demonstrated how higher order vertebrates such as river otters can respond to PAC exposure through altered steroid hormone metabolism with effects felt at the population level. While PAC exposure is one of many stressors experienced by river otters in the AOSR, fecal steroid hormone profiles were consistent with reproductive failure. Future work should consider increasing sample sizes to capture river otter populations that are not impacted by petrogenically-derived PACs in order to evaluate the robustness of our conclusions.

5.4. References

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Appendix A.

Supplementary Information

Supplementary Tables

Table S1- Wet weight normalized PAC data in biota pooled across sites

	N	Mean	Median	Min	Max	Std.Dev.	Detection Rate
Snails							
Parent LMW	3	17.85	1.03	0.57	51.94	29.52	100%
Alkylated LMW	3	75.23	2.70	0.00	223.00	127.98	66%
Parent HMW	3	4.12	3.62	0.00	8.73	4.39	66%
Alkylated HMW	3	61.23	48.87	0.00	134.82	68.26	66%
Parent HET	3	0.24	0.00	0.00	0.72	0.42	33%
Alkylated HET	3	75.03	0.51	0.00	224.60	129.53	66%
Lake White Fish							
Parent LMW	8	0.37	0.00	0.00	1.88	0.67	38%
Alkylated LMW	8	2.88	1.33	0.00	13.63	4.45	88%
Parent HMW	8	0.29	0.00	0.00	2.30	0.81	13%
Alkylated HMW	8	2.37	0.38	0.00	8.61	3.61	50%
Parent HET	8	0.00	0.00	0.00	0.00	0.00	0%
Alkylated HET	8	0.06	0.00	0.00	0.46	0.16	13%
Northern Pike							
Parent LMW	10	2.05	0.84	0.00	13.51	4.13	70%
Alkylated LMW	10	23.69	8.66	0.00	150.06	45.25	90%
Parent HMW	10	0.00	0.00	0.00	0.00	0.00	0%
Alkylated HMW	10	11.26	3.41	0.00	47.56	16.61	70%
Parent HET	10	0.00	0.00	0.00	0.00	0.00	0%
Alkylated HET	10	0.85	0.00	0.00	4.85	1.82	20%
Walleye							
Parent LMW	13	9.64	0.36	0.00	95.99	26.61	54%
Alkylated LMW	13	6.11	3.54	0.00	26.06	7.18	92%
Parent HMW	13	1.68	0.00	0.00	20.58	5.69	15%
Alkylated HMW	13	30.29	0.31	0.00	197.97	62.99	54%
Parent HET	13	0.00	0.00	0.00	0.00	0.00	0%
Alkylated HET	13	1.99	0.00	0.00	10.13	3.00	46%

River Otter							
Parent LMW	47	0.65	0.00	0.00	5.29	1.24	49%
Alkylated LMW	47	3.30	1.12	0.00	40.31	7.61	96%
Parent HMW	47	0.07	0.00	0.00	3.65	0.47	6%
Alkylated HMW	47	0.31	0.00	0.00	7.38	1.09	23%
Parent HET	47	0.00	0.00	0.00	0.00	0.00	0%
Alkylated HET	47	0.10	0.00	0.00	1.26	0.30	15%

Table S2- Count of samples included in analysis (n=81) from each site. SAGD= steam-assisted gravity drainage, SMA= surface mineable area, PAD= Peace Athabasca Delta. The snail sample was a pool of snails collected at the site.

	Biota				
Site	Snails	Lake Whitefish	Northern Pike	Walleye	River Otter
Burnt Lakes	1	2	1		9
SAGD	1	3	3	6	13
SMA	1		2	2	17
PAD		3	4	5	8

Supplementary Figures

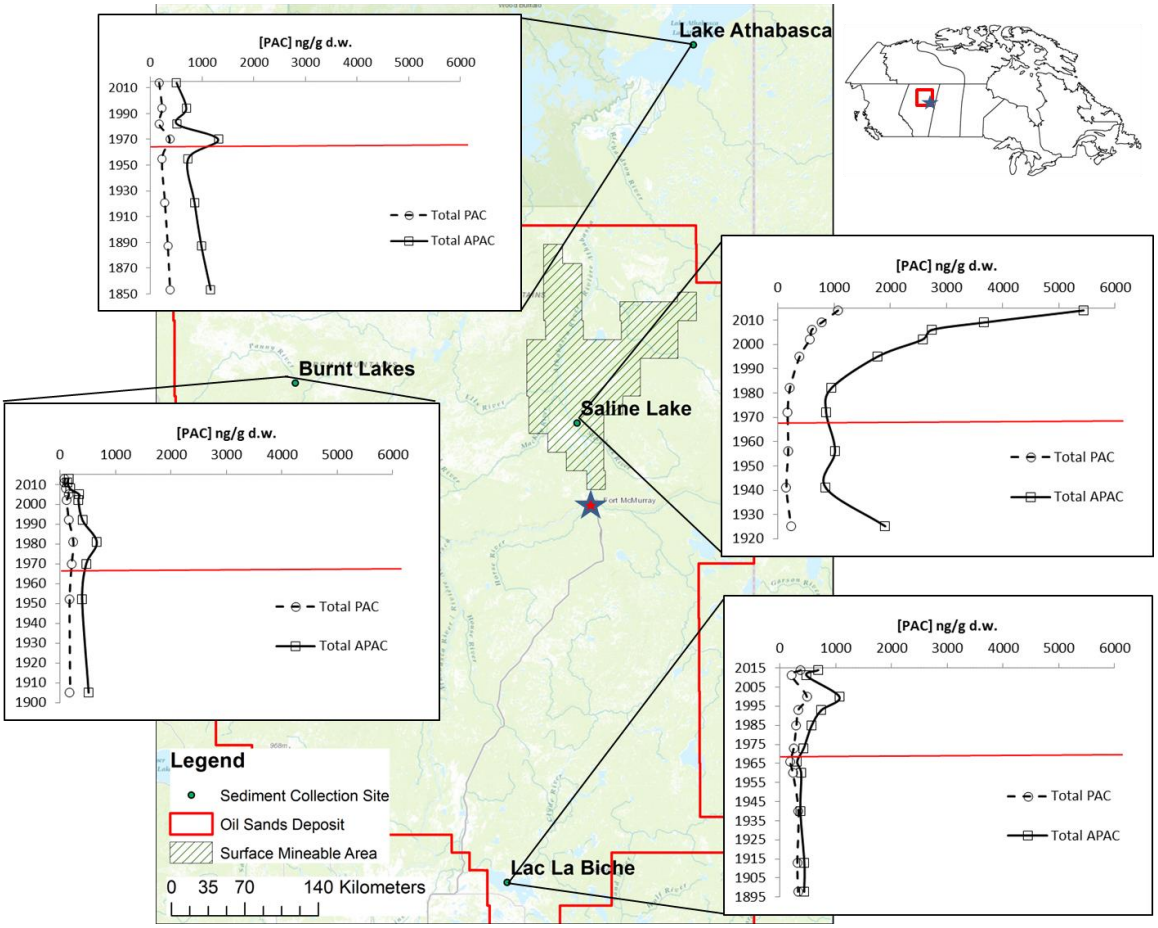

Figure S1- Dry weight normalized PAC data in sediment cores

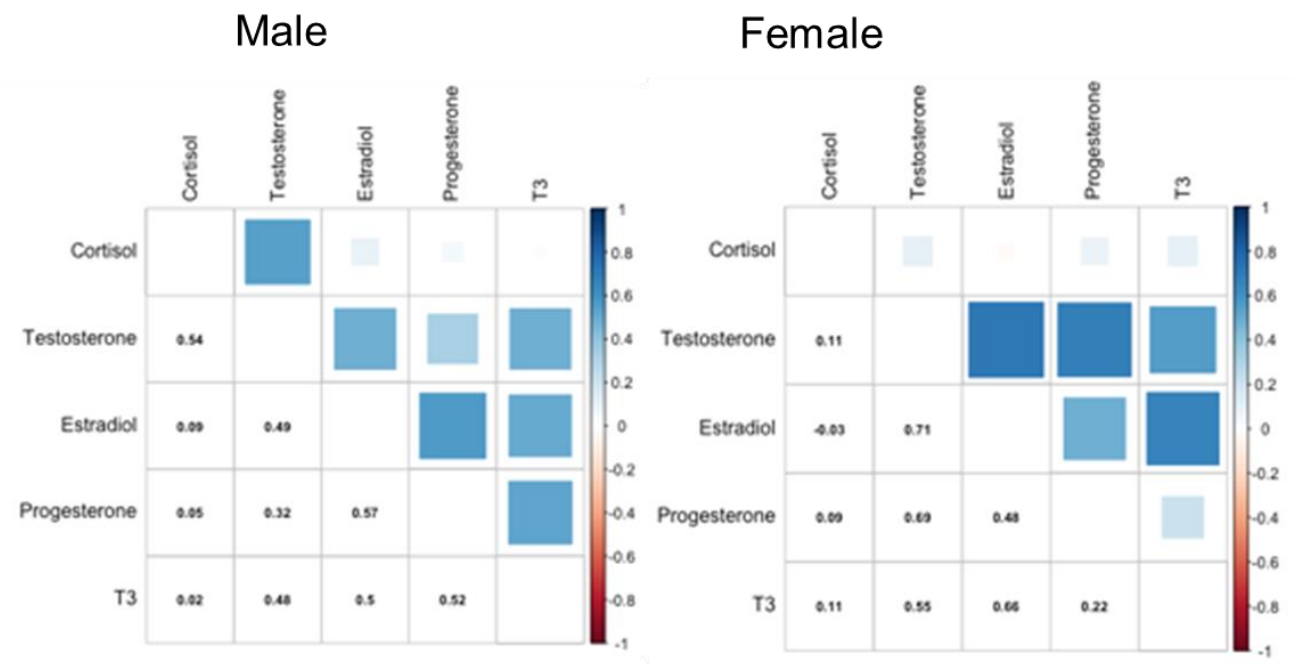

Figure S2- Correlations between hormones for males and females. The r value can be found on the lower diagonal, and a visual representation of the r values can be found on the upper diagonal.