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Polycyclic aromatic hydrocarbons in caribou, moose, and wolf scat samples from three areas of the Alberta oil sands



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ABSTRACT

Impacts of toxic substances from oil production in the Alberta oil sands (AOS), such as polycyclic aromatic hydrocarbons (PAHs), have been widely debated. Studies have been largely restricted to exposures from surface mining in aquatic species. We measured PAHs in Woodland caribou (*Rangifer tarandus caribou*), moose (*Alces americanus*), and Grey wolf (*Canis lupus*) across three areas that varied in magnitude of *in situ* oil production. Our results suggest a distinction of PAH level and source profile (petro/pyrogenic) between study areas and species. Caribou samples indicated pyrogenic sourced PAHs in the study area previously devastated by forest fire. Moose and wolf samples from the high oil production area demonstrated PAH ratios indicative of a petrogenic source and increased PAHs, respectively. These findings emphasize the importance of broadening monitoring and research programs in the AOS.

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1. Introduction

The Alberta oil sands (AOS) is the third largest proven reserve of oil in the world underlying 142,200 square kilometres (km²) of land (Alberta, 2013a). The bitumen (thick, heavy crude oil), which is refined for consumer and industrial use, is mixed with sand and water deep below the earth's surface. Extraction of these oil reserves has required *in situ* drilling technologies; steam assisted gravity drainage (SAG-D) is the predominant form of extraction in the AOS, particularly south of Fort McMurray. Steam mobilizes and separates the bitumen deep below the surface (e.g., 350 m), and only the bitumen is removed and transported. *In situ* technologies are advertised by the Canadian Association of Petroleum Producers to have lower environmental impact than surface mining as measured by greenhouse gas emissions, land use, water use, and tailings ponds (CAPP, 2014). Although this technology disturbs less land on the surface, it has been shown to have a spatial footprint equivalent to surface mining when considering increased landscape fragmentation due to seismic lines, access roads, pipelines, and well sites (Jordaan et al., 2009). The perceived environmental and public health impact of surface mining activity, north of Fort

McMurray, has been the focus of controversy with demands to reduce or halt development in the AOS (Gibbins, 2010; Kelly et al., 2009; Timoney and Lee, 2009). However, few independent studies are available on the environmental and public health impacts of oil exploration and bitumen extraction using *in situ* methods south of Fort McMurray.

Attention surrounding the AOS stems from the perception that these reserves are integral to North American energy security coupled with local to international campaigns highlighting negative impacts on human, wildlife, and ecological health (Gibbins, 2010). Of particular concern are exposures to polycyclic aromatic hydrocarbons (PAHs), a diverse groups of compounds found in bitumen, among other sources such as forest fires and diesel exhaust, known to be carcinogens and mutagens, and rank in the top 10 hazardous substances by the United States Agency for Toxic Substances and Disease Registry (ATSDR, 2013; Baird et al., 2005; Culp et al., 1998; Xue and Warshawsky, 2005). PAHs can be resistant to degradation making it imperative for future planning initiatives to be grounded on an understanding of the impacts of PAH exposure on the maintenance of biodiversity and ecologic processes. The Woodland caribou of northern Alberta are listed as threatened (Alberta Woodland Caribou Recovery Team, 2005), yet the extent and routes of PAH exposure and potential long-term impacts on this population are poorly understood. A better understanding of PAH exposures in large terrestrial mammals is also imperative for the health of tribal and recreational hunters.

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Sustainable hunting communities in the AOS, particularly First Nations who have retained treaty rights to hunt and fish in their traditional homeland for subsistence (Alberta, 2013c), depend on the health of this ecosystem to ensure their game is pollutant free. Common big game in Alberta includes moose, caribou, and deer. Moose in particular are the most frequently consumed traditional food among First Nation groups (McLachlan, 2014).

Caribou feed largely on lichen, when available (Naughton, 2012; Wasser et al., 2011). Lichen can readily absorb airborne contaminants; including the lightweight and volatile 2–4 ring PAHs (Belis et al., 2011; Blasco et al., 2007, 2006; Kelly and Gobas, 2001), potentially exposing caribou to trace contaminants from long-range transport of the compounds. Kelly et al. (2009) calculated over 11,000 metric tons of airborne particles, consisting mostly of bitumen, as far as 50 km from upgrading facilities. PAHs can persist in environmental compartments, such as lichen, due to their slow growth and longevity (Blasco et al., 2006). As such, caribou may be exposed to PAHs from land disturbance or forest fire for a prolonged period after the event. Moose feed largely on riparian vegetation, particularly Red Oster Dogwood and various willow species that depend upon moist soils that may facilitate absorption of PAHs through ground water (Naughton, 2012). Although the uptake of PAH compounds varies by properties of the plant, the pollutants, and environmental conditions (Belis et al., 2011; Migaszewski et al., 2002), water and sediment is a well-established contamination source of PAHs in the AOS (Akre et al., 2004; Hall et al., 2012; Headley et al., 2001; Kurek et al., 2013; Wayland et al., 2008). PAH exposure may also occur through atmospheric transport and deposition on the woody vegetation. Exposure to PAHs in carnivores could occur through ingestion of PAH-laden prey. An evaluation of prey hair in wolf scat samples, analysed as part of this same ongoing work, demonstrated over 90% of the winter diet in wolves consisted of deer, moose, and caribou; 24% of wolf diet was comprised of moose, 11% of caribou, and 65% of deer after adjusting for biomass (Wasser et al., 2011). Similar findings have been reported from a separate study in Northeastern Alberta, including sites that overlap our study area (Latham et al., 2011).

Our study measured polycyclic aromatic hydrocarbons in scat (fecal) samples collected over a 2500 km² area with varying degrees of *in situ* oil production activity, providing an unprecedented opportunity to non-invasively monitor levels of PAHs in three terrestrial species with markedly different resource utilizations. We evaluated three wide-ranging terrestrial mammals that inhabit the AOS, Woodland caribou (*Rangifer tarandus caribou*), moose (*Alces americanus*), and Grey wolf (*Canis lupus*). This allowed us to compare levels across species that have markedly distinct dietary differences, life histories, and associated routes of exposure for a comprehensive look at potential contamination of different environmental resources.

2. Material and methods

2.1. Study area

Scat samples from caribou, moose and wolf were collected over a 2500 km² area south of Fort McMurray, spanning three caribou ranges within the East Side of the Athabasca River caribou herd that differed in oil production activity and fire histories (Fig. 1). The extent of oil development and exploration was evaluated using the Alberta Energy Regulation's List of Wells in Alberta, Statistical Report 37 (AER, 2014), including all reported wells starting in 1957 up through and including 2009 in locations defined using the Alberta Township System (ATS) (ATS, 2014). The Egg Pony (EP) range, located in meridian 4 including ranges 7–13 and townships

77–83, was the site of the greatest amount of oil development and exploration activity during our winter 2009 study period. The EP area had 4 active wells and 34 wells drilled and cased. The Wiau range, to the immediate south of the EP, located in meridian 4 including ranges 9–12 and townships 76–77, included oil exploration and extraction activity at a lower intensity than the EP, with no active wells and 7 wells drilled and cased. In addition, an extensive fire in 2002 (the House River fire) burned over 238,000 ha of the range (Alberta, 2013b) including the entire Wiau sampling area. No other fires on record in the three study areas since 1996 were larger than 20,000 ha. The Algar range, approximately 160 km to the north of the EP and 70 km west-southwest of Fort McMurray, along the Athabasca River, located in meridian 4 including ranges 13–17 and townships 84–87, had no active or capped wells on record although historical exploration may have occurred, as evidenced by survey lines visible on Google Earth (Google Inc; Mountain View, CA). These 3 major geographic areas sampled allowed for a spectrum of oil development and exploration activity to be represented and evaluated, even in the absence of a true reference site.

2.2. Sample collection

Scat samples were located by trained detection dogs between mid-December of 2008 and mid-March of 2009, as part of the ongoing work on the impacts of AOS development on caribou, moose, and wolf (Wasser et al., 2011). The survey included 36 cells in the EP, four cells in the Wiau, and eight cells in the Algar. DNA was extracted from all scat samples and used to confirm the species, sex, and individual identities (Wasser et al., 2011). Available funding restricted the number of samples that could be analysed in each species. We aimed to randomly select one fecal sample from 15 genetically unique individuals of each sex per species and area. However, the number of samples analysed from each sex and species varied across study areas based on sample detections in the field. For example, only one wolf sample was collected in the Algar (not analysed) and no male caribou or female wolf samples were collected in the Wiau.

2.3. Laboratory and analytical methods

PAH extraction, purification, and quantification methods were developed and performed at the University of Washington, Seattle using modified procedures from other studies (Cochran et al., 2012; Forsberg et al., 2011; Mazéas and Budzinski, 2005; United States Environmental Protection Agency, 1996). All samples were spiked with a surrogate standard (Acenaphthylene-d10) (Accustandard; New Haven, CT USA) prior to extraction to monitor extraction recovery and for internal standard quantification using isotope dilution methods. All samples were previously lyophilized (Wasser et al., 2011). For caribou and moose samples, 1.0 g of dry fecal material was saponified (alkaline decomposition of lipids) using 9.0 mL of 1 M potassium hydroxide:ethanol (80% ethanol:20% Millipore water) then extracted with 10.0 mL of hexane. Due to matrix interference and poor recovery of internal standards in the wolf sample, 0.35 g of dry fecal material was first extracted in 10.0 mL of hexane, then saponified as described above. This modification served to saponify only the extract, not other components of the fecal bulk (e.g., prey hair). The final extract solution for all species received 20.0 mL of magnesium sulfate and sodium chloride (4:1). The resulting hexane extract was evaporated to approximately 1.0 mL, and loaded onto a 500 mg Discovery-aminopropyl Solid Phase Extraction cartridge (Supelco; Bellefonte, PA USA). The target compounds were eluted using 12.0 mL hexane. The final extract was evaporated to 0.5 mL and stored in the

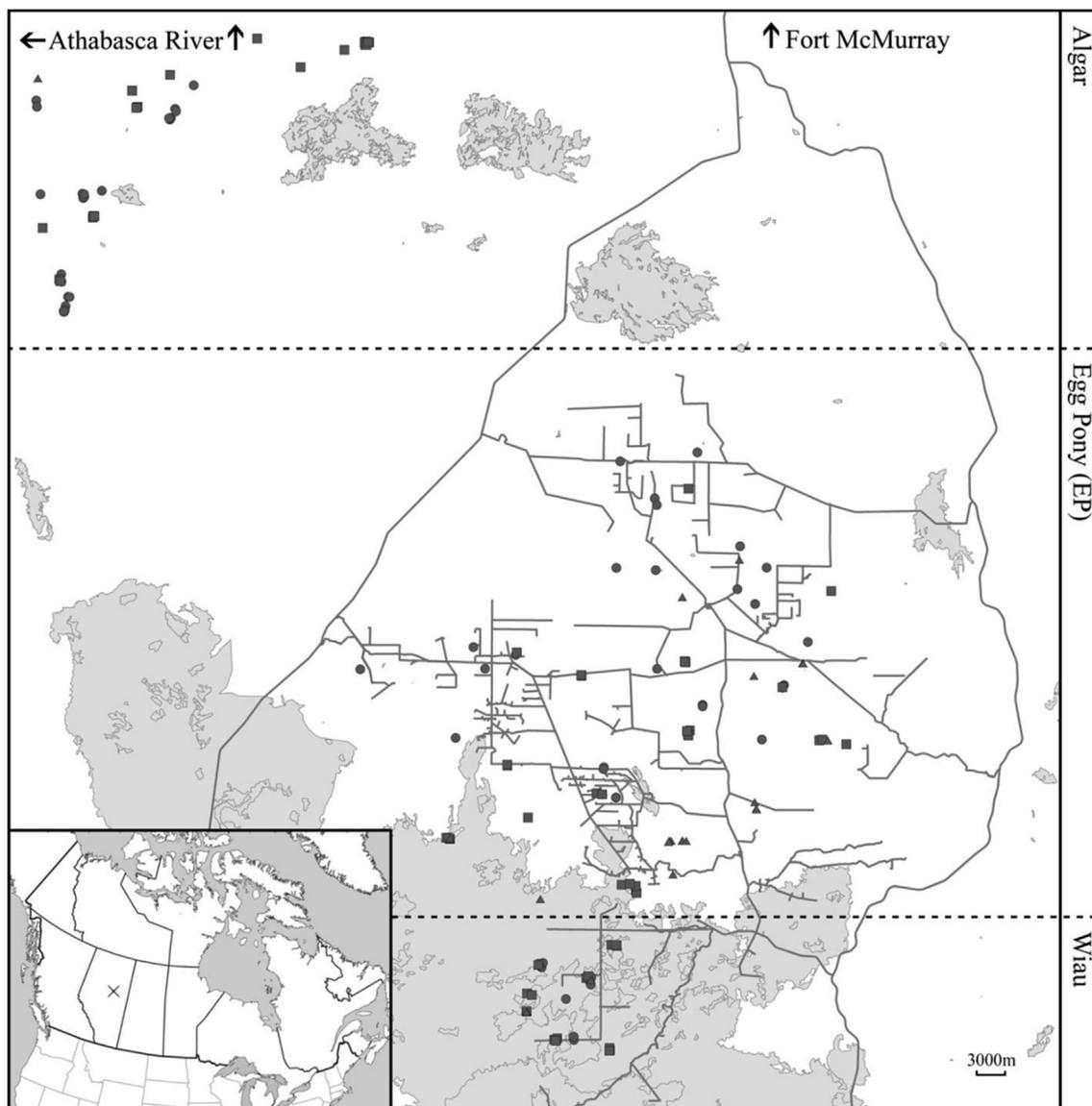


Fig. 1. Fecal samples from caribou (●), moose (■) and wolf (▲) were collected over a 2500 km² area across three caribou ranges within the East Side of the Athabasca River caribou herd, separated by dashed horizontal lines (top: Algar; middle: Egg Pony; bottom: Wiau), that differed in level of *in situ* oil production activity and fire history. Grey shaded area in main figure denotes historical wildlife. Grey solid line denotes primary and secondary access roads. Inset map denotes sampling area (X) within Alberta, Canada.

dark at $-20\text{ }^{\circ}\text{C}$ until analysis.

2.4. Analysis

All sample extracts and standards were analysed using an Agilent 7890A gas chromatograph coupled with an Agilent 5975C mass spectrometer (GCMS). The MS system was operated with electron ionization (70 eV) and in select ion monitoring mode. The instrument was fitted with a DB-5MS column (30 m length, 0.25 μm film thickness, 0.25 mm i.d.) (Agilent, Santa Clara, CA). The injection port was operated in splitless mode, fitted with a glass liner, and delivered 2 μL by auto-injection. The carrier gas was helium. Chromatographic separation was achieved using an initial temperature of 50 $^{\circ}\text{C}$ with a 4 min hold, ramping to 100 $^{\circ}\text{C}$ at a rate of 10 $^{\circ}\text{C}$ per minute, immediately followed by ramping to 145 $^{\circ}\text{C}$ at a rate of 5 $^{\circ}\text{C}$ per minute, to 150 $^{\circ}\text{C}$ at 1 $^{\circ}\text{C}$ per minute, then to 250 $^{\circ}\text{C}$ at 5 $^{\circ}\text{C}$ per minute, with a final ramp to 310 $^{\circ}\text{C}$ at 10 $^{\circ}\text{C}$ per minute with a 4 min hold. The total run time per sample was 53 min.

2.5. Quantification and quality assurance

Concentrations of individual PAH compounds were determined by internal standard quantification. A stock solution of 27 parent (unsubstituted) and alkylated (derivatives with one to four methyl-group substitutions) PAHs was prepared by combining a custom mix of priority pollutant PAHs and individual PAHs [Accustandard (New Haven, CT USA), Chiron (Trondheim, Norway), and Sigma (St. Louis, MO USA)] in hexane. Each analytical batch (by species) included an eight-point standard calibration curve at the beginning and end of each run, a calibration check standard every 20 samples, a laboratory procedure blank for each extraction group, three repeat quantification samples to test instrument accuracy and precision, three repeat extraction samples to test laboratory accuracy and precision, and solvents blanks (hexane). The regression coefficient of the calibration curve slope was >0.999 for all target compounds. The calibration check standard response factors were checked for each series of samples analysed, and were typically

$\pm 10\%$ the expected values. PAH recoveries were calculated by comparing the relation of the surrogate standard signal intensity in the sample to that in the standards (defined to be 100%). Mean percent recovery by species were: 81% (standard deviation (sd); 13) for caribou, 84% (sd; 19) for moose, and 73% (sd; 16) for wolf. One moose and one caribou sample were excluded from the final analysis due to a recovery greater than 3 standard deviations from the mean. Three target analytes were excluded from all analyses due to co-elution of artifact compounds in the matrix (1,2-dimethylnaphthalene, 1,8-dimethylfluorene, and acenaphthalene). Any measured responses in the procedure lab blanks were subtracted from the responses measured in samples from the same extraction group. Duplicate sample extractions were typically $\pm 10\%$ the expected values. All sample concentrations are reported as nanogram per gram dry fecal weight (ng/g dw).

Method detection limits were generally less than 1.0 ng/g dw, except for naphthalene (2.3 ng/g dw), phenanthrene (2.8 ng/g dw), 1,4,6,7-tetramethylnaphthalene (4.0 ng/g dw), and chrysene/benzo[a]pyrene (3.2 ng/g dw). Results reporting less than the method detection limit were replaced with the detection limit divided by the square root of two; non-detections were replaced with zero.

2.6. Statistical analysis

A number of PAH metrics were selected *a priori* to evaluate differences between study areas by species using general linear model marginal mean estimates. The *a priori* PAH metrics included total (Σ)PAHs, a cumulative measure of all naphthalenes, fluorenes, phenanthrenes, and anthracenes (parent and alkylated compounds), as well as flouranthene and pyrene. The metric Σ Petro-PAHs included parent and alkylated compounds of three of the five PAHs commonly used for identification of petrogenic source PAHs: naphthalenes, fluorenes, and phenanthrenes (Wang and Brown, 2009); Σ Parent and Σ Alkyl included just the parent or alkylated compounds, respectively. The Petroleum Index evaluated the quotient of Σ Parent divided by Σ Alkyl. A greater presence of parent compounds is indicative of pyrogenic source PAHs due to cleaving off of carbon-based methyl groups during combustion. Alkylation of a parent compound is a characteristic of a petrogenic source PAH. Sex was considered as a covariate where possible and was retained if significant in the models. An effect was considered significant when $p < 0.05$.

A number of PAH ratios have been successfully applied for distinguishing between petrogenic and pyrogenic PAH signatures. PAH ratios are useful for minimizing any concentration effect and are self-normalizing since variations due to instrument operating conditions, final sample volume, or matrix effects are minimized. Many PAH ratios involve pairs with the same molar mass and similar physiochemical properties for likeness when undergoing environmental fate processes. Fluoranthene and pyrene are 4-ring PAH isomers that are commonly used to distinguish between combustion (pyrogenic) and petroleum (petrogenic) sources (fluoranthene/(fluoranthene + pyrene) (F/FP)) (Carls, 2006; Jautzy et al., 2013; Jiang et al., 2009; Liu et al., 2009; Soclo et al., 2000; Wang and Brown, 2009). Crude oil generally contains more pyrene than fluoranthene, however fluoranthene is more stable than pyrene in high-temperatures such as combustion (Wang and Brown, 2009). A ratio of F/FP < 0.4 was considered indicative of petrogenic source PAHs. The F/FP ratio is considered a sensitive measure of type of combustion; a ratio of 0.4–0.5 was considered characteristic of fossil fuel combustion and >0.5 an indicator of biomass combustion (pyrogenic). An additional PAH ratio used for this study evaluated the presence of alkylation of phenanthrene, an indication of petrogenic source, in relation to parent phenanthrene ($(1\text{-methylphenanthrene} + 2\text{-methylphenanthrene})$

phenanthrene; MP/P). A ratio >1.0 was considered an indicator of a petrogenic PAH source, and <1.0 an indicator of a mixed PAH source. This ratio has been successfully applied to characterize pyrogenic or petrogenic hydrocarbon contaminants in other studies (Kim et al., 2008; Notar et al., 2001; Zakaria et al., 2002). Proportion of samples meeting petrogenic or pyrogenic criteria for both PAH ratios were compared between study areas by species using a chi-square test, or Fisher's exact test when appropriate. An effect was considered significant for $p < 0.05$. All statistics were performed using SAS 9.3 (Cary, NC).

3. Results

Final analysis included 24 target analytes from 62 caribou, 69 moose, and 29 wolf fecal samples from three study areas in the AOS (Fig. 1; Table 1).

3.1. Caribou

Woodland caribou samples demonstrated a statistically higher level of Σ PAHs (ng/g dw) in the fire-impacted Wiau study area (geometric marginal mean (mean), 95% confidence interval (CI); 115.4, 102.2–130.3) compared to the Algar (93.6, 84.7–103.3) and the EP study areas (97.6, 89.4–106.6) (Fig. 2). Elevated mean Σ Parent levels of 28.3 ng/g dw were also demonstrated in the Wiau study area (95% CI, 24.8–32.3) compared to a mean of 23.9 ng/g dw in the Algar and 26.9 ng/g dw in EP areas (95% CIs, 21.4–26.6 and 24.4–29.5, respectfully). This increase in PAHs, particularly of parent PAHs, suggest that the source of the increase PAH levels in samples from the Wiau are from a combustion source (e.g., fire or diesel exhaust). PAHs levels evaluated by sex were not significantly different in the EP or Algar areas. Lack of male caribou samples in the Wiau prohibited testing for sex differences.

The fluoranthene/(fluoranthene + pyrene) (F/FP) ratio in caribou samples from the Wiau area was also characteristic of a pyrogenic PAH source (Fig. 3). The F/FP ratio is considered a sensitive measure of type of combustion; for this study a ratio >0.5 was considered an indicator of biomass combustion (pyrogenic). The mean ratio in the Wiau (mean, 95% CI; 0.66, 0.55–0.77) was statistically higher than the mean ratio in the EP area (0.46, 0.38–0.54) (data not shown). The cross-plot of diagnostic ratios (Fig. 3) demonstrate that significantly more samples from the Wiau study area (12/14 samples; 86%) had PAH ratios characteristic of a pyrogenic source based on a F/FP ratio >0.5 and a methylphenanthrenes/phenanthrene (MP/P) ratio <1.0 , indicative of a non-petrogenic source, compared to the other study areas (EP, 41% of samples; Algar, 52% of samples) ($\chi^2 = 7.61$, $p < 0.02$). This pyrogenic PAH source in the Wiau caribou samples likely has a biomass combustion source origin from the House River forest fire that engulfed the area in 2002.

3.2. Moose

Moose samples did not demonstrate a difference in Σ PAHs between the three study areas indicating their level of exposure between study areas was similar. However, a statistically higher mean level of Σ Alkyl PAHs (ng/g dw) was demonstrated in the Algar study area (i.e., control site) (mean, 95% CI; 32.6, 27.1–39.3) compared to the EP (23.1, 19.7–27.1) and Wiau areas (22.5, 18.6–27.2) (Fig. 2) indicating a petrogenic exposure in the Algar study area [refer to discussion section]. This finding was dominated by alkylated naphthalenes (Table 1). Sex was not significant in any model.

The PAH ratio of F/FP demonstrated a predominant petrogenic origin of PAHs (F/FP < 0.4) in the moose samples from the EP study

Table 1

Median and standard deviation (sd) of PAH target compounds (ng/g dw) in fecal samples from caribou, moose, and wolf from three areas of the oil sands region of Alberta, Canada.

Target compound	Caribou (n, male/n, female)						Moose (n, male/n, female)						Wolf (n, male/n, female)			
	Algar (11/10)		EP (12/15)		Wiau (0/14)		Algar (7/13, 1 unk)		EP (14/14)		Wiau (6/14)		EP (10/10)		Wiau (8/0)	
	Median	sd	Median	sd	Median	sd	Median	sd	Median	sd	Median	sd	Median	sd	Median	sd
NPH	11.0	3.5	12.6	6.9	11.8	2.9	5.7	16.5	19.0	11.9	5.6	3.1	25.4	17.3	21.9	9.0
MN2	10.7	3.5	10.0	3.4	10.1	7.4	13.0	8.3	5.1	5.7	5.7	2.7	16.5	9.7	10.2	5.2
MN1	7.7	2.2	7.1	2.3	8.1	4.7	8.9	5.3	2.0	3.5	3.0	1.9	8.2	6.2	5.0	3.0
DMN	3.0	1.1	2.6	1.1	2.6	1.1	3.5	1.6	3.6	1.0	3.3	0.8	3.8	2.1	3.3	1.7
TriMN	1.9	1.1	2.0	1.3	1.8	1.8	1.2	1.1	0.6	0.9	0.5	0.7	2.1	6.0	0.1	1.2
TetMN	0.7	0.6	0.2	0.5	0.2	0.3
PHN	10.1	2.6	11.3	4.9	13.6	4.4	3.2	2.0	2.1	1.4	4.3	1.7	10.8	21.6	3.1	2.6
MP2	4.0	1.5	3.7	2.3	5.2	1.5	0.1	0.6	2.0	0.9	0.2	1.5	1.2	8.0	1.7	2.7
MP1	2.9	1.6	3.0	2.3	5.8	1.8	0.7	1.4	1.8	1.8	0.6	1.7	2.5	5.9	0.5	0.6
DMP	1.0	1.5	2.1	2.0	1.0	1.7	1.0	0.4	1.1	0.4	0.9	0.6	0.7	2.4	0.0	0.1
TriMP	0.3	3.7	0.5	0.2
TetMP	1.8	1.7	1.7	1.0
DBT	0.7	0.4	0.8	0.4	0.4	0.4	0.3	0.3	0.1	0.6	0.3	0.6	0.6	3.2	0.2	0.0
FLO	1.3	0.7	1.7	1.0	1.9	1.0	1.4	0.9	0.8	0.5	0.8	0.6	2.5	4.0	1.4	1.0
FL1	1.4	1.0	1.7	1.4	1.9	2.2	1.7	1.0	1.9	1.8	2.3	2.3	2.6	5.5	1.3	1.0
CHR/B[a]A	8.5	4.1	10.0	4.2	12.5	3.9	0.4	0.3	1.2	0.6	0.2	0.6	6.1	2.1	6.0	0.4
ACY	1.2	0.9	1.6	1.2	1.4	1.0	0.0	0.2	0.0	0.1
ANT	0.0	1.7	0.0	0.9	0.0	0.2	0.9	7.7	0.0	15.5	0.4	10.1	1.5	4.3	0.4	0.4
MA2	0.0	0.1	0.0	0.3	0.0	0.2	0.7	0.4	0.1	2.5	1.0	0.3	0.6	0.8	0.6	0.2
MA9	16.3	7.4	15.4	6.7	17.0	3.8
MA23	0.1	1.6	0.7	1.5	2.1	1.6	0.4	0.7	0.4	1.5	0.4	0.5	0.5	4.4	0.9	0.2
MA910	1.4	1.3	1.9	1.2	2.8	1.3	1.2	3.7	1.1	2.6
FLU	1.3	1.9	2.2	2.8	5.6	2.7	0.3	0.3	0.7	0.5	0.5	0.3	0.7	4.6	0.0	0.5
PYR	1.5	1.3	2.0	1.6	2.8	1.4	0.3	1.4	2.0	1.5	0.6	0.9	2.3	6.2	0.0	0.2

NOTE:Period (.) denotes target compound not detectable. Abbreviations: naphthalene (NPH); 1-methylnaphthalene (MN1); 2-methylnaphthalene (MN2);1,6-dimethylnaphthalene (DMN); 2,3,5-trimethylnaphthalene (TriMN); 1,4,6,7-tetramethylnaphthalene (TetMN); phenanthrene (PHN); 1-methylphenanthrene (MP1); 2-methylphenanthrene (MP2); 3,6-dimethylphenanthrene (DMP); 1,2,5-trimethylphenanthrene (TriMP); 1,2,6,9-tetramethylphenanthrene (TetMP); dibenzothiophene (DBT); fluorene (FLO), 1-methylfluorene (FL1); Chrysene/benz[a]anthracene (CHR/B[a]A; co-eluted); acenaphylene (ACY); anthracene (ANT); 2-methylanthracene (MA2); 9-methylanthracene (MA9); 9,10-dimethylanthracene (MA910); 2,3-dimethylanthracene (MA23); fluoranthene (FLU); and pyrene (PYR).

area (i.e., area of most intensive oil production) (Fig. 3). The mean ratio in the EP (mean, 95% CI; 0.23, 0.16–0.29) was statistically lower than the mean ratio in the Wiau (0.43, 0.35–0.50) and in the Algar (0.44, 0.36–0.51) study areas (data not shown). Similarly the PAH ratio of MP/P, used to indicate petrogenic source when >1.0, also demonstrated a predominant petrogenic origin of PAHs in the moose samples from the EP study area. The mean MP/P ratio in the EP (mean, 95% CI; 2.22, 1.36–3.60) was statistically higher than the mean ratio in the Wiau (0.48, 0.27–0.86) and in the Algar (0.40, 0.23–0.70) study areas (data not shown). The cross-plot of PAH ratios (Fig. 3) demonstrated an apparent cluster of moose samples, with 86% (24/28 samples) of samples from the EP area having PAH ratios indicative of a petrogenic origin (i.e., F/FP < 0.4 and a ratio of

MP/P > 1.0). This proportion of petrogenic PAH samples in the EP area, based on PAH ratios, was significantly greater ($\chi^2 = 28.13$, Fisher's exact test p-value <0.001) than the proportion of petrogenic samples from the Wiau (6/20 samples, 30%) or Algar (3/21 samples, 14%) study areas.

3.3. Wolf

The Grey wolf scat samples exhibited a statistically higher mean level of Σ PAHs (ng/g dw) in the EP area (mean, 95% CI; 109.7, 84.4–142.5) compared to the Wiau area (64.4, 42.6–97.4) (Fig. 2). We did not have wolf samples from the Algar study area. The elevated level of PAHs was dominated by alkylated PAHs, indicative

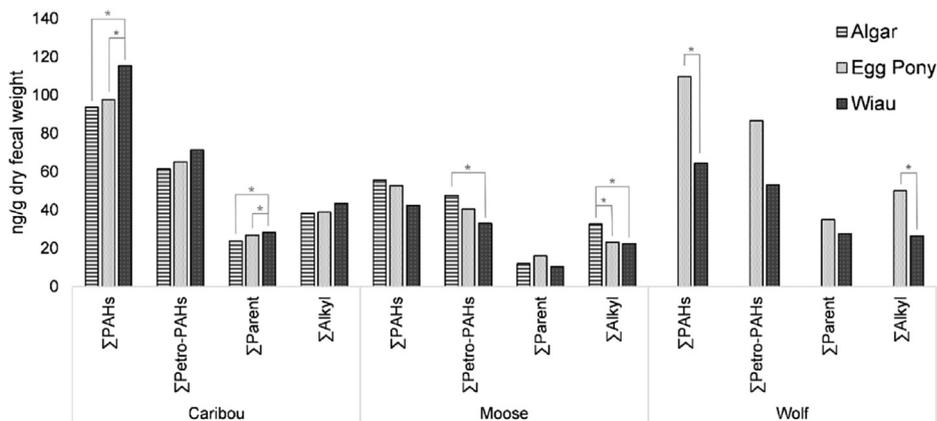


Fig. 2. Geometric marginal means (ng/g dw) of PAH metrics in fecal samples from caribou, moose, and wolf from three areas of the Alberta oil sands. *p < 0.05

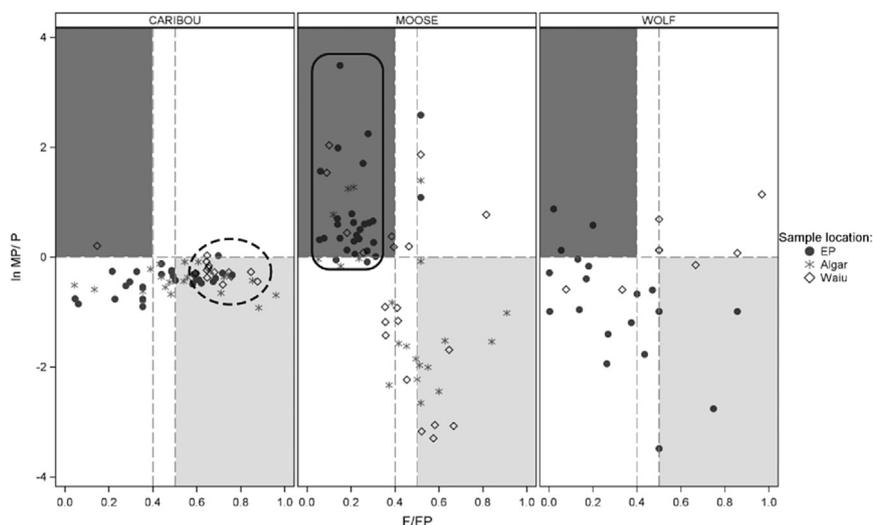


Fig. 3. PAH ratios for distinguishing pyrogenic vs petrogenic PAH source. Ratios indicative of petrogenic sources fall above the horizontal line ($\ln MP/P > 0$) and to the left of the two vertical lines ($F/FP < 0.4$), samples between the two vertical lines suggest fossil fuel combustion sources ($F/FP 0.4\text{--}0.5$), and samples below the horizontal line ($\ln MP/P < 0$) and to the right of the two vertical lines ($F/FP > 0.5$) indicate pyrogenic (biomass combustion) or mixed sources. The left panel demonstrates ratios for caribou, the middle panel for moose, and the right panel for wolf. The dashed circle denotes the cluster of samples from the Wiau study area, and the solid line rectangle denotes the cluster of samples from the EP study area.

of a petrogenic source, demonstrated by an increase in mean levels of \sum Alkyl in the EP (mean, 95% CI; 50.0, 38.4–65.1) compared to the Wiau (26.5, 17.5–40.2) (Fig. 2). A decreased Petroleum Index, \sum Parent PAH compounds divided \sum Alkyl compounds, in the EP area (mean, 95% CI; 1.75, 1.61–1.89) compared to the Wiau (2.05, 1.81–2.32) also suggest a predominance of alkylated PAHs in this area (data not shown; $p < 0.05$). Lack of female wolf samples in the Wiau prohibited testing for sex differences.

The PAH ratios suggested a combination of petrogenic and pyrogenic exposure sources in the wolf samples. The ratio of F/FP was significantly lower in the EP study area (mean, 95% CI; 0.31, 0.19–0.43) compared to the Wiau area (mean, 95% CI; 0.55, 0.37–0.73) (data not shown), indicating a petrogenic PAH source. In total, 12 of the 20 (60%) wolf samples from the EP area, compared to 2 of 8 (25%) Wiau samples, had F/FP ratios < 0.4 , indicative of a petrogenic origin (Fig. 3). However, a mean MP/P ratio of 0.41 (95% CI; 0.26–0.63) in wolf samples from the EP area indicate non-petrogenic or mixed exposure (data not shown); only three samples had a petrogenic MP/P ratio greater than 1.0.

4. Discussion

The ability to measure PAHs in scat samples of multiple terrestrial wildlife species with distinct dietary and resource utilization differences allowed us to monitor PAH levels in these species across an expansive landscape. Our results demonstrate that PAH exposures vary between study areas by species. The need to balance the maintenance of a healthy thriving ecosystem with competing economic objectives is crucial as bitumen production in the AOS is projected to more than double between 2008 and 2018 (Alberta, 2013a). These findings illustrate the importance of further scientific investigation and including toxicant levels and their environmental consequences in monitoring programs of *in situ* drilling technologies in the AOS. The noninvasive sampling method applied in this study is ideally suited for such investigations owing to the availability of wildlife scat in the environment and its relative ease of collection over large geographic areas.

Multiple lines of evidence indicate a pyrogenic PAH signature in caribou samples from the Wiau area where a 238,000 ha fire occurred in 2002. These include an increased level of parent PAHs

($p < 0.05$) and a predominance of pyrogenic PAH ratios (86%; $p < 0.02$) compared to the other study areas. This finding may be reflective of increased PAH levels in lichen from the fire stricken area, particularly since lichen is the preferred food source of caribou and a well-established reservoir of PAHs. However, lichen samples were not collected for this study so the actual level of PAHs in the lichen available to caribou is not known. By contrast, moose and wolf samples from the EP study area, where the most intensive oil extraction and exploration activity occurred, each had evidence of exposure to petrogenic PAHs compared to the other study areas. Most moose samples from the EP study area (86%; 24/28 samples) demonstrated PAH ratios suggestive of a petrogenic source; this was significantly greater than the petrogenic source PAH ratios from the other study areas ($p < 0.001$). PAH exposures may have occurred through absorption of ground water by their preferred diet of riparian vegetation, deposition on woody vegetation from atmospheric transport of these compounds, or another unexplained source. Wolf samples from the EP study area demonstrated statistically increased levels of total ($p < 0.05$) and alkylated ($p < 0.05$) PAHs, suggestive of a petrogenic source, that were not evident in samples from the other study areas. Ingestion of PAH-laden prey is a possible source of PAH exposure in wolves. However, other routes of exposure such as social grooming or cleaning of paws after walking through contaminated snow-pack could provide additional sources of exposure. Both moose and wolf species showed evidence of mixed source exposures. These sources may have been better differentiated by a larger sample size and sampling of presumed environmental exposures.

The presumed petrogenic sources of PAHs in the AOS include crude oil exposure from natural deposits, seepage through geographic strata, potential leaks or spills, or increased exposure to natural deposits through disturbance of the landscape or the addition of steam into bitumen rich areas as part of exploration and extraction processes. Presumed pyrogenic (high-heat combustion) sources of PAHs would be historical forest fires, petroleum combustion from a related increase in road development and motorized traffic in the area, or industrial emissions. Mixed source profiles were evident in all species, likely influenced by a combination of the potential PAH sources listed above. Surprisingly, the cumulative levels of PAHs in moose were highest in samples from the Algar

compared to other areas. The Algar is an area of habitat restoration with no active or capped oil wells on record, dating back to 1957 (AER, 2014; COSIA, 2014). These increased levels may reflect the relative proximity of the Algar study area to both the intensive surface mining activities north of Fort McMurray (70 km east-northeast of the Algar area), nearness to the Athabasca River (Fig. 1) where oil seepages make their way through geographic strata into the watershed, or other unknown sources of PAH exposure. The mixed PAH source signature in the caribou samples across the EP and Algar areas could reflect a combination of smaller and/or unreported forest fires, volatilization of compounds (subsequently absorbed by the lichen) from continuously running diesel trucks (in the EP) or disturbance of the landscape, or sources outside of the study area brought in by atmospheric transport. The mixed source exposure in the wolf may be a reflection of their diverse prey, including moose (petrogenic), caribou (pyrogenic), deer, beaver, and snow shoe hare. Other unexplained PAH sources are also possible and warrant further study.

Past studies have demonstrated bioaccumulation of persistent organic pollutants (POPs) [e.g., polychlorinated biphenyls (PCBs)] in top-level arctic carnivores, such as the wolf and other arctic wildlife (Fisk et al., 2005; Kelly and Gobas, 2001; Sormo et al., 2006). PAHs are more susceptible to metabolism, the biologic half-life of benzo[a]pyrene in plasma from rats has been demonstrated to be less than 6 h (Ramesh et al., 2001), and are less lipophilic (i.e., decreased affinity for long-term storage in fat) compared to POPs, thus do not bio-magnify as readily in the food web. Nonetheless, with declines in use of POPs, PAHs are reportedly beginning to emerge as the dominant contaminant in the arctic food web despite their more acute exposure (Laender et al., 2011). The present study measured PAHs in wolf scat samples at higher levels in areas of increased oil extraction activity. These measures are likely the result of recent PAH transfer, possibly through the consumption of PAH-laden food sources. Prey hair analyses of wolf scat from this same project, corroborated by findings from a separate study, demonstrated that 24% of the wolf winter diet consist of moose (Latham et al., 2011; Wasser et al., 2011). Moose are also preferred game of First Nation communities proximal to the AOS, as well as common game of recreational hunters (McLachlan, 2014). The extent that increased PAH levels in wolves is acquired from its moose prey is not known at this time. Nonetheless, our reported petrogenic PAH profile in the moose samples from the area of increased oil exploration and extraction could have public health implications related to tribal and recreational hunting in areas proximal to active oil production.

Few comparable studies exist on contamination levels and adverse effects of PAHs in wild terrestrial mammals (McLachlan, 2014). Health effects of PAHs are often assessed through the concentration of 5-ring or larger compounds, such as benzo[a]pyrene, that are well studied and considered “known animal carcinogens” (ATSDR, 2013); however, these compounds were not detectable in the samples from this study. Reproductive toxicities have been associated with exposures to benzo[a]pyrene in rats (Arafa et al., 2009) as well as 3-ring PAHs, such as phenanthrene, in Japanese medaka (*Oreozias latipes*) (Hornig et al., 2010), marine calanoid copepod (Bellas and Thor, 2007), and enchytraeids (Sverdrup et al., 2002). Research on the health impacts of exposure to alkylated PAH compounds is limited, although a study evaluating alkyl-phenanthrene demonstrated an increase in embryo abnormalities (*Oryzias latipes*) compared to unsubstituted phenanthrene (Turcotte et al., 2011). Regardless of whether current PAH exposures in terrestrial wildlife in the AOS are at levels likely to cause carcinogenic effects or reproductive toxicities, sublethal exposures and indirect effects due to disrupted trophic interactions (Peterson et al., 2003), or toxic effects induced by ultraviolet radiation

exposure (i.e., sunlight) such as the generation of reactive oxygen species that cause membrane damage (Arfsten et al., 1996; Incardona et al., 2012; Yu, 2002), may still be detrimental.

Further sampling from subsequent years and presumed environmental exposure sources (e.g., lichen) would markedly improve our understanding of the extent and impact of PAH exposure among the study species. Additional sampling would be especially informative given the projected increases in petroleum exploration and development activity (Alberta, 2013a). It is recommended that future sampling efforts include white-tailed deer since they are the preferred wolf prey (Wasser et al., 2011) and are increasing in numbers in the AOS (Latham et al., 2011). Previous studies have demonstrated that PAH metabolites are present in feces at higher levels than the parent compounds (Bouchard and Viau, 1998; Hillenweck et al., 2008; Jonsson et al., 2004; Ramesh et al., 2001). This study focused on comparing the measurements of parent compounds within species and between ranges; thus our results are a conservative estimate of the actual PAH levels. The within species comparisons ensured that reported findings between study areas were not due to species differences in bioavailability, metabolic capacity, or gut microflora leading to increased metabolites that would inflate the level of measured PAHs. Including a spectrum of PAH metabolites may be considered in future studies.

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