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Elevated exposure, uptake and accumulation of polycyclic aromatic hydrocarbons by nestling tree swallows (*Tachycineta bicolor*) through multiple exposure routes in active mining-related areas of the Athabasca oil sands region

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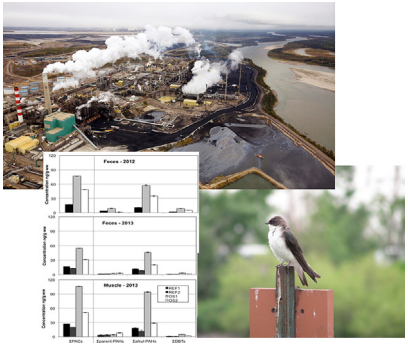
HIGHLIGHTS

- Exposure of nestling tree swallows to PACs examined in the Athabasca Oil Sands
- A complex mixture of 41 PACs detected in chicks' feces and accumulated in muscle
- Levels of PACs higher in birds, air, water at mining-related than reference sites
- Major sources of PAC exposure for regional birds were diet, then air and water.
- Non-lethal sampling (feces) is useful to characterize avian exposure to PACs.

GRAPHICAL ABSTRACT



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ABSTRACT

In the Athabasca Oil Sands (OS) Region, the exposure (by air, water, diet), uptake and deposition of polycyclic aromatic compounds (PACs), including parent and alkylated hydrocarbons (PAHs) and dibenzothiophenes (DBTs), was assessed in nestling tree swallows (*Tachycineta bicolor*) at mining-related (OS1, OS2) and reference (REF) sites. The OS sites did not receive oil-sands processed waters (OSPW) and were ≥60 km from the reference sites. Most of the 42 PACs (≤98%) were detected in all matrices. Swallows at the OS sites were exposed to higher air and water concentrations of individual PAC congeners, ΣPACs, Σparent-PAHs, Σalkyl-PAHs and ΣDBTs. Compared to reference nestlings (ΣPACs: 13–27 ng/g wet weight (ww)), PACs were significantly higher in OS nestlings (31–106 ng/g ww) that also accumulated higher concentrations of major PAHs (i.e., naphthalene, C1-naphthalene, C2-naphthalene, C1-fluorenes, C2-fluorenes, C1-phenanthrenes) measured in 60% of nestlings. Uptake and deposition of PAHs in the birds' muscle was related to diet (δ^{15}N: C1-naphthalenes, C2-naphthalenes,

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Alkylated PAHs
Dibenzothiophenes
Athabasca Oil Sands

C1-fluorenes), water (C1-phenanthrenes), and air through inhalation and feather preening (C1-fluorenes), but fecal concentrations were not well explained by diet or environmental concentrations. While PAH concentrations were much higher in muscle than feces, they were highly correlated ($p \leq 0.001$ for all). Thus feces may represent a non-lethal method for characterizing PAH exposure of birds, with muscle characterizing accumulation and sources of PAH exposure. Tree swallows in the Athabasca OS Region are exposed to many PACs, accumulating higher concentrations when developing in close proximity to mining activity through diet, aerial deposition and mining-impacted freshwater sources (e.g., wetlands).

1. Introduction

The Athabasca Oil Sands Region is a vast area (142,000 km^2) with a large reserve of bitumen in northern Alberta and Saskatchewan, Canada (CCAoP, 2016a), and is the third largest known oil reserve globally (Alberta Go, 2012). It has been an area of active mining (904 km^2) for approximately 50 years (CCAoP, 2016a). In 2015, 3.98 million barrels of crude oil per day were extracted, which despite the decline in production in 2016, is projected to increase to 5.45 million barrels by 2030 (CCAoP, 2016b). Water is required to extract bitumen (~4 barrels of water per barrel of crude oil), resulting in large quantities of Oil Sands process-affected water (OSPW) that are held in tailings ponds until they are reclaimed (Reviewed in: (Giesy et al., 2010; Parajulee and Wania, 2013)). Polycyclic aromatic compounds (PACs) and other contaminants derived from mining activities enter the environment through the seepage of OSPW into regional waters, and by long range transportation and aerial deposition of emissions and suspended particles (Kelly et al., 2009; Zhang et al., 2016), particularly within 50 km of active mining sites (Kelly et al., 2009).

Although many PACs are naturally present in this bitumen rich environment, PACs have increased significantly (by 2.5 to 23 times) in lake sediment cores since industrial extraction began, indicating a direct impact on the environment from mining activity (Kurek et al., 2013). Few studies have detailed the composition of PACs in either air (including snowpack) or water (including sediment) in the Oil Sands Region, but a complex mixture of PACs has been identified, including polycyclic aromatic hydrocarbons (PAHs), alkylated PAHs (alkyl-PAHs) and dibenzothiophenes (DBTs) and their alkylated forms (Kurek et al., 2013; Schuster et al., 2015). In the Athabasca Oil Sands Region, the PAC profile is dominated by alkyl-PAHs (Kurek et al., 2013; Schuster et al., 2015) that are largely of petrogenic origin (i.e., associated directly with recent mining activity), unlike parent PAHs that are also released during the combustion of fossil fuels and forest fires (reviewed in (Lundin et al., 2015)). The occurrence of DBTs in the PAC profile in air throughout the OS Region further substantiates the petrogenic origin of this PAC mixture ((Schuster et al., 2015) and references therein).

Despite the prolonged intensive extraction of bitumen in the Athabasca Oil Sands Region, few studies have examined the deposition and accumulation of PACs by vertebrates near active mining in the region. The highest concentrations of PAHs were measured in fish (Ohiozebau et al., 2016) and mammals (Lundin et al., 2015) inhabiting areas near or within oil-impacted areas compared to those inhabiting more remote locations. Birds, such as tree swallows (*Tachycineta bicolor*), also have the potential to accumulate high concentrations of PAHs from sediment through their diet (Custer et al., 2017). Tree swallows primarily consume insects whose larvae develop in aquatic sediment (Mengelkoch et al., 2004), and in the OS Region, PAHs have accumulated in sediments (Smits et al., 2000; Wayland et al., 2008) and aquatic insects (Wayland et al., 2008). Exposure to sufficient concentrations of PAHs may cause a broad spectrum of health effects in vertebrates, from altering molecular and physiological processes, to modifying hepatic and immune function, increasing deformities, reducing reproductive success and growth, and causing acute toxicity in birds (reviewed in (Albers, 2006; Leighton, 1993)). The risks of exposure and possible effects of other types of PACs are largely unknown although some alkyl-PAHs have mutagenic effects and may be partially responsible for toxic effects of environmental mixtures of PAHs (reviewed in (Baird et al., 2007)). Tree swallows breeding on reclaimed wetlands in the Oil Sands Region have demonstrated increased detoxification activity (measured by hepatic ethoxyresorufin O-dealkylase (EROD)) activity (Cruz-Martinez et al., 2015), changes in thyroid hormone concentrations (Gentes, McNabb et al., 2007), increased parasitism (Gentes, Whitworth et al., 2007) and increased mortality during inclement weather (Gentes et al., 2006) compared to birds at reference sites.

With the growing evidence that PACs are increasing in the environment as a result of mining activities in the Athabasca OS Region (Kelly et al., 2009; Kurek et al., 2013), that aquatic-emerging insects accumulate PACs in regional wetlands (Wayland et al., 2008), and that PACs have the potential to cause adverse effects in vertebrates, it is increasingly important to characterize and understand the potential exposure and accumulation of PACs by birds breeding in this region. As part of the present study, airborne PAH concentrations were characterized during the reproductive season of tree swallows in 2012 (Cruz-Martinez et al., 2015). However to date, no studies have investigated the exposure, deposition and accumulation of PACs by birds breeding in close proximity to Oil Sands industrial activities, nor examined the environmental sources (i.e., air, water, diet) of exposure to PACs for birds in this region.

In the current study, our objectives were to determine whether birds nesting in close proximity to active mining-related activities in the Athabasca Oil Sands Region were exposed to and accumulated greater concentrations of PACs compared to those breeding at reference sites; we used tree swallows as an avian model as they have the potential to accumulate parent- and alkyl-PAHs (Custer et al., 2017; Wayland et al., 2008). We also sought to compare and determine PAC concentrations between tissue and fecal samples in the same individuals so as to develop a non-lethal method to characterize avian exposure, uptake and sequestering of PACs, for future research and monitoring purposes. Our final objectives were to: i) characterize PAC and DBT concentrations in environmental matrices (i.e., air, water) relative to those in the nestlings' feces and muscle, ii) model the birds' exposure, uptake and deposition of PACs from the environment, and iii) to determine the importance of each environmental matrix and the birds' diet, as potential routes of exposure to PACs for the birds. Inhalation of contaminants, including air contaminants measured in the Athabasca OS Region, have adversely affected thyroid function in captive birds (Fernie et al., 2016). Because the diet of tree swallows is largely comprised of aquatic-emerging aerial insects known to accumulate PACs in regional wetlands (Wayland et al., 2008) and elsewhere (Custer et al., 2017), we hypothesized that diet would be another important source of exposure to PACs for birds. We further hypothesized that air may also contribute to body burdens, as PACs have been detected in the air in the OS Region (Schuster et al., 2015) and inhalation is a common route of exposure to contaminants. To the best of our knowledge this is the first study characterizing PAC contaminants in birds inhabiting OS-impacted sites, and it is the first study to examine whether environmental PAC concentrations contribute to internal body burdens in terrestrial breeding vertebrates.

2. Materials and methods

2.1. Study sites and field collections

This study was conducted in 2012 and 2013 with all appropriate permits and was approved by the Canadian Council of Animal Care. As previously described (Cruz-Martinez et al., 2015), colonies of nest boxes for breeding tree swallows were established at four study sites, with 15 to 34 nest boxes per site, immediately adjacent to on-site water bodies not receiving industrial runoff or OSPW. Two colonies were north of Fort McMurray in the surface mining area of the region within 5 km of active mining, extraction and processing (e.g., open mine pits, processing plants) (Fig. 1). The first study site near active mining-extraction activities (OS1) was undergoing reclamation (57° 17′ 54.10″, 111° 23′ 24″), while the second site (OS2) was previously reclaimed (56° 59′ 19.80″, 111° 34′ 4.80″) with minimal deposition from nearby upgraders. Additional tree swallow colonies were established at two reference sites, REF1 in 2012, and REF2 in 2013, south of Fort McMurray (Fig. 1) that were outside of the surface mining zone, more than 100 km from regional industrial mining-extraction-processing activities.

Nest monitoring began on May 20th in both years, and nests were monitored daily throughout the reproductive period with the exception of when the birds were incubating their clutch of eggs. At hatching, nestlings were considered as 0 days old, and two nestlings from each brood were randomly selected at approximately 14 d of age. Fecal samples from each individual were voluntarily collected directly into cryovials that were immediately stored in liquid nitrogen until analysis. Procedures relating to blood sampling, euthanasia, and full necropsies of nestlings are detailed in (Cruz-Martinez et al., 2015). Briefly, the nestlings were anesthetized in an enclosed container using a 50 mL falcon tube containing isoflurane soaked cotton balls, and euthanized by cervical dislocation. Immediately afterwards, birds were dissected. In 2013, 1 g

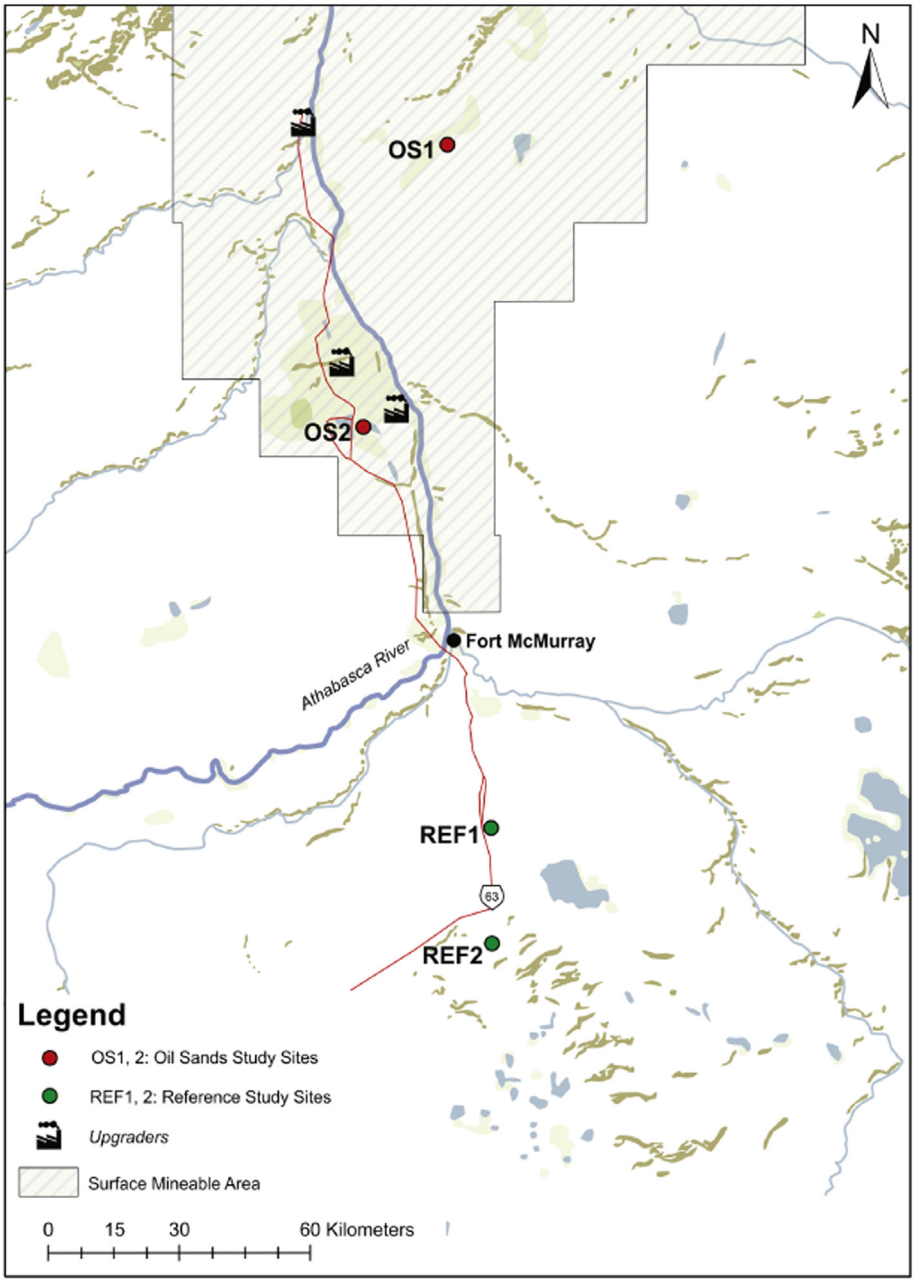

Fig. 1. Colonies of tree swallows breeding in nest boxes at four different study sites, the reference sites REF1 and REF2, and at two Oil Sands mining-related sites in the Athabasca Oil Sands Region of Alberta, Canada. Nest boxes were established at REF1, OS1 and OS2 in 2012, and at REF2 in 2013.

of pectoral muscle was collected, placed in a 2 mL cryovial, then stored in liquid nitrogen until transferred to a − 80 °C freezer. Fecal and muscle samples were used to analyze PACs. Dorsal feathers were collected for stable isotope analysis and stored individually in manila coin envelopes until the time of analysis.

PAC concentrations were also assessed in water samples collected by grab sampling from the surface of the water immediately adjacent to the nest boxes at each study site (1 sample per site). The water samples were collected once (4 July 2013) during the nestling period because of logistical constraints. Grab samples were collected in 1-L amber glass bottles at the shore of each onsite freshwater body. The amber glass bottle was submerged approximately 30 cm below the water surface with the cap removed under water, filled to capacity, then returned to the surface with the cap reattached. Water samples were kept at 1–4 °C from the point of collection until extraction and analysis.

As previously described (Cruz-Martinez et al., 2015), a polyurethane foam (PUF) passive air sampler was installed at each colony for 40 d from when the birds first returned to breed until the last nestlings were euthanized at the site, providing an integrated measure over the duration of their reproductive period. When the PUF passive air samplers were installed, field blanks were collected at each site (the PUF was exposed to air for 1 min, then stored in an airtight jar).

Weather variables and ambient temperatures for the region were collected from the closest weather station in Fort McMurray (Environment and Climate Change Canada, Climate ID: 3,062,696; 56°39′ 04.000″ N, 111°12′48.000″ W). Mean temperatures (°C) and total precipitation (mm) were calculated for the 14 d nestling period of each nestling.

2.2. Analysis of PAC concentrations in nestlings

Analytical details for passive air samples (Cruz-Martinez et al., 2015), water samples and stable isotopes (Fernie et al., 2017), are based on previously developed methodologies and presented in the SI. The Chen Laboratory (DC) analyzed the PACs and DBTs in bird samples, separately by tissue type. The reference standards of target PAH analytes were purchased from Absolute Standards, Inc. (Hamden, Connecticut), ChemService Inc. (West Chester, Pennsylvania), Chiron AS (Trondheim, Norway), and Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA). Deuterated PAH standards (d-PAH) were purchased from Absolute Standards, Inc. and combined to make a Surrogate Standard mixture. They included d_8-naphthalene, d_{10}-2-methylnaphthalene, d_8-acenaphthylene, d_{10}-acenaphthene, d_{10}-fluorene, d_{10}-phenanthrene, d_{10}-anthracene, d_{10}-fluoranthene, d_{10}-pyrene, d_{12}-benzo(a)anthracene, d_{12}-chrysene, d_{12}-benzo(b)fluoranthene, d_{12}-benzo(k)fluoranthene, d_{12}-benzo(a)pyrene, d_{12}-perylene, d_{12}-indeno(1,2,3-cd)pyrene, d_{14}-dibenzo(a,h)anthracene, and d_{12}-benzo(g,h,i)perylene. Diatomaceous earth (DE) and sodium sulfate (10–60 mesh), purchased from Fisher Scientific (Hanover Park, IL), were treated in a muffle furnace at 600 °C overnight (>12 h) and cleaned with dichloromethane (DCM) prior to use. Isolute® silica sorbent (average pore size: 60 Å) was purchased from Biotage Inc. (Charlotte, NC, USA) and baked at 130 °C (>6 h) prior to analysis. High-performance liquid chromatography (HPLC) grade solvents were purchased from Fisher Scientific.

2.3. Preparation of nestling samples

Feces or pectoral muscle from each bird were homogenized with DE. After spiking with the surrogate standard mixture containing 25 ng each of the d-PAHs, the sample was subject to accelerated solvent extraction or ASE (Dionex ASE 350, Sunnyvale, CA, USA) employing two 5-min extraction cycles with DCM at 100 °C and 1500 psi, then prepared for instrumental analysis of PACs by gas chromatography mass spectrometry (GC–MS). The resulting extract was subject to gravimetric determination of lipid content by using 10% of the extract. The remaining extract was cleaned and separated on a 2-g Isolute® silica solid phase extraction (SPE) column packed into a 6 mL Superclean™ glass cartridge (Sigma-Aldrich, St. Louis, MO, USA). The Isolute SPE sorbent was pre-cleaned with DCM via sonication to remove potential contamination. The packed SPE column was pre-washed with 10 mL hexane (HEX) to condition the silica gel sorbent. After the sample was loaded, the first fraction was eluted with 3 mL HEX and was discarded since there were no analytes contained in this fraction. The second fraction contained target PAHs and d-PAHs and was eluted with 11 mL 60:40 HEX:DCM. The latter fraction was concentrated to approximately 200 μL and transferred to an insert tube in a GC vial. Internal standard d_{14}-dibenzo(a,i)pyrene (50 ng; Toronto Research Chemicals, Toronto, Canada) was added prior to instrumental analysis.

The separation and quantification of target PAHs, surrogate and internal standards, was performed on an Agilent 7890 gas chromatograph (GC; Agilent Technologies, Palo Alto, CA) coupled to a single quadrupole mass analyzer (Agilent 5977A MS) in electron impact (EI) mode. The column used for GC–MS analysis was a 30 m HP-5MS column (0.25 mm i.d., 0.25 μm, J&W Scientific, Agilent Tech.). The injector was operated in pulsed-splitless mode, held at 280 °C. Initial oven temperature was held at 50 °C for 1 min, and then ramped to 280 °C at 8 °C/min and held for 15 min. The quantification and confirmation of each target compound was achieved via selected ion monitoring (SIM) for its characteristic ions under EI ionization mode. Quantification was based on calibration curves built from five standard solutions containing individual PAH or d-PAH concentrations ranging from 0.2 to 200 ng/mL and internal standard concentration of 200 ng/mL.

2.4. Quality assurance and control (QA/QC) of nestling samples

Several measures were employed for QA/QC purposes including spiking experiments, analysis of Standard Reference Materials (SRMs), process of procedural blanks, and examination of surrogate standard recoveries. Known amounts of PAC analytes were spiked with DE and analyzed using the methodology described above to evaluate analyte recoveries throughout the analysis. A spiked sample was processed with every two batches ($N = 5$ per batch) of authentic samples. The mean (±standard deviation) of recoveries of individual PAH analytes from spiking experiments ranged from 75 ± 5% to 94 ± 5%. A NIST SRM 1974c sample (muscle tissue; *Mytilus edulis*) was processed along with every three batches of authentic samples. The determined PAC concentrations ranged from 75 ± 4% to 107 ± 4% of the certified concentrations. A procedural blank was run with every 5 samples to check for laboratory contamination, which revealed a total PAC concentration of 0.3–1.35 ng/g in blanks. Recoveries of surrogate standards (i.e., d-PAHs) ranged from 70 ± 4% to 91 ± 6% in authentic samples. All PAC concentrations were corrected based on surrogate standard recoveries and reported as ng/g ww after subtracting blank contamination. The limit of detection (LOD), estimated as an analyte response that was 5 times the standard deviation of the noise during instrumental analyses, ranged from 0.05 to 0.15 ng/g ww.

2.5. Statistical methods

Values of PACs below the detection limit were assigned a value of zero. Initially, data were evaluated for normal distribution and homogeneity of variance using Kolmogorov-Smirnov and Levene's tests, and log-transformed when data failed to meet these criteria. We used Analysis of Covariance (ANCOVA) to identify differences in fecal PACs among sites and years (two-factor), and in muscle PACs among sites (one-factor), with brood, Julian hatch date and sex of the nestlings as covariates, followed by post-hoc Least Significant Difference (LSD) tests. Kruskal-Wallis tests ($\delta^{15}N$) and ANCOVAs with Julian lay date as a covariate ($\delta^{13}C$) were used to identify significant differences among sites in feather stable isotopes in 2013. Pearson or Spearman's correlation analyses were used to determine possible correlations between fecal and muscle

concentrations of the major PACs, and *t*-tests were used to determine if there were significant differences between tissue types and years.

To identify the factors that may have contributed to the variation in PAC concentrations of nestling muscle or feces in 2013 (the only year with a complete data set), a series of Generalized Linear Mixed Models (GLMMs) were conducted and ranked using Akaike's Information Criterion corrected for small sample sizes (AIC_C). Model sets were created for each group of PAC congeners, as well as for the individual major PACs that were quantified in ≥60% of individual nestlings. The data were categorized by site (OS1, OS2, REF1, REF2) followed by nest box within site to account for nestling-relatedness and similarity in diet. The ΔAIC_C and Akaike's weights (w_i) were calculated; the latter measure signifying the likelihood that a model is the best model relative to the other models tested. Models were constructed with up to three of the following fixed factors that may have influenced the PAC concentrations: nestling sex, body mass, hatching date (to account for possible seasonal variation), stable isotopes (diet), water and air concentrations of the same PACs, mean ambient temperature and/or total precipitation for the 14 d prior to tissue collection. Only models for which all factors were significant (i.e., confidence intervals did not cross zero) were included in the model selection. Linear regressions were also conducted to determine the adjusted R^2, and to estimate the proportion of the variation explained by each model (on uncategorized data). Statistics were conducted using IBM SPSS© 23 with a significance level of *p*-value ≤ 0.05.

3. Results

3.1. Nestlings: PAC profile and site differences

Most PACs were detected in the nestlings: six major PAHs were detected in at least 60% of the birds, specifically naphthalene, C1– and C2-naphthalenes, C1– and C2-fluorenes, and C1-phenethrenes, and ∑alkyl-PAHs dominated the PAC profile in muscle and feces (Table 1, Figs. 2, 3). Of the individual parent and alkylated PAHs, C2-naphthalenes were present in the highest concentrations followed by C1-phenanthrenes (Fig. 3). The ΣDBTs were measured at lower concentrations in the nestlings (Fig. 2).

Fecal and muscle concentrations of the major PAHs, ΣPAHs, Σalkyl-PAHs, and ΣDBTs were significantly different among the four study sites (*F*-values ≤ 63.33; *p-values* ≤ 0.05) (Figs. 2, 3), with no statistical effects of brood, nestling sex, and hatch date evident. Without exception, PAH concentrations in muscle and feces were statistically similar between the two reference sites, but nearly all PAHs significantly differed between OS1 and OS2 (LSD *p-values* ≤ 0.02) (Figs. 2, 3). Nestlings at OS1 had significantly higher concentrations (feces, muscle) of nearly all major PAHs, ΣPACs, Σalkyl-PAHs, and ΣDBTs, than nestlings at OS2, or the reference sites (Figs. 2, 3) (LSD *p-values* ≤ 0.02). Similarly, OS2 nestlings had significantly higher concentrations (feces, muscle) of individual PAHs, ΣPACs, Σalkyl-PAHs, and ΣDBTs (feces), compared to the birds at REF1 (LSD *p-values* ≤ 0.001) and REF2 (LSD *p-values* ≤ 0.03) (Figs. 2, 3). Fecal concentrations of ΣPACs, Σalkyl-PAHs and ΣDBTs significantly differed between years among the study sites (site*year interactions: *F*-values ≥ 16.35, *p-values* ≤ 0.001), and were higher in 2012 than 2013 at OS1 and OS2 (*p-values* ≤ 0.01) with no evidence of temporal changes at REF1 (Fig. 4).

3.2. Relationships between fecal and muscle PAH concentrations in nestlings

In 2013, the PAH concentrations in feces and muscle of all chicks were significantly and highly correlated (*r-values* < 0.97; $p \le 0.001$). Muscle concentrations were significantly greater than fecal concentrations for all individual major PAHs, ΣPAHs and Σalkyl-PAHs ($t < 4.89$; *p-values* < 0.02) but not ΣDBTs (Fig. 5). There was a greater difference between tissue types for the OS nestlings than the reference nestlings (site*tissue type interaction: *p-values* ≤ 0.007): in the OS chicks, muscle had higher concentrations than feces of naphthalene, C2-fluorenes, C1-phenanthrenes, ΣPAHs, Σalkyl-PAHs, and ΣDBTs, suggesting greater deposition and accumulation of these PAHs and DBTs in the OS chicks.

Table 1
Concentrations (ng/g ww) of the major and summed PACs measured in nestling tree swallows at reference sites (REF1, REF2) and at two sites in close proximity to Oil Sands mining-related activities (OS1, OS2) in Alberta, Canada (2012 and 2013 combined). Arithmetic means (mean) and standard errors about the mean (SE) are presented. $N = 74$ fecal samples; $N = 70$ muscle samples; we report % Detected for 2012, 2013 (fecal) and 2013 (muscle).

	% Detected	REF1		REF2		OS1		OS2	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
2012, 2013									
Fecal concentrations									
naphthalene	89, 96	0.76	0.06	0.70	0.07	1.30	0.07	1.5	0.16
C1-naphthalenes	97, 98	1.60	0.14	1.66	0.16	3.33	0.26	3.24	0.32
C2-naphthalenes	100	3.83	0.24	3.04	0.31	15.0	0.92	8.18	0.93
C1-fluorenes	89, 96	0.76	0.07	0.89	0.07	1.64	0.14	1.54	0.08
C2-fluorenes	83, 82	0.42	0.08	0.58	0.13	2.70	0.21	2.01	0.17
C1-phenanthrenes	80, 93	1.49	0.25	0.91	0.08	6.61	0.93	2.99	0.54
ΣPACs	100	17.5	0.91	13.4	0.85	62.1	3.58	38.9	2.99
Σparent-PAHs	100	2.8	0.43	1.67	0.31	5.25	0.91	4.15	0.68
Σalkyl-PAHs	100	9.39	0.79	7.98	0.46	41.3	3.21	20.8	2.35
ΣDBTs	78, 91	1.27	0.22	0.79	0.21	5.15	0.98	3.24	0.63
2013									
Muscle concentrations									
naphthalene	86	1.08	0.27	0.98	0.18	1.85	0.17	2.75	0.42
C1-naphthalenes	100	2.41	0.38	2.29	0.23	3.79	0.34	4.26	0.49
C2-naphthalenes	99	6.21	0.70	4.32	0.60	18.5	1.07	8.17	1.06
C1-fluorenes	97	0.94	0.09	1.12	0.10	2.13	0.26	2.12	0.15
C2-fluorenes	87	0.43	0.11	0.74	0.14	3.22	0.32	2.09	0.17
C1-phenanthrenes	99	3.68	0.79	1.56	0.17	28.9	4.09	6.28	2.49
ΣPACs	100	27.5	2.12	20.6	1.25	104	8.89	54.8	7.00
Σparent-PAHs	100	3.6	0.58	3.81	0.63	4.17	0.61	8.08	1.48
Σalkyl-PAHs	100	18.8	2.06	12.1	0.86	91.6	9.27	34.0	7.18
ΣDBTs	96	1.16	0.3	0.83	0.25	4.65	0.65	2.32	0.94

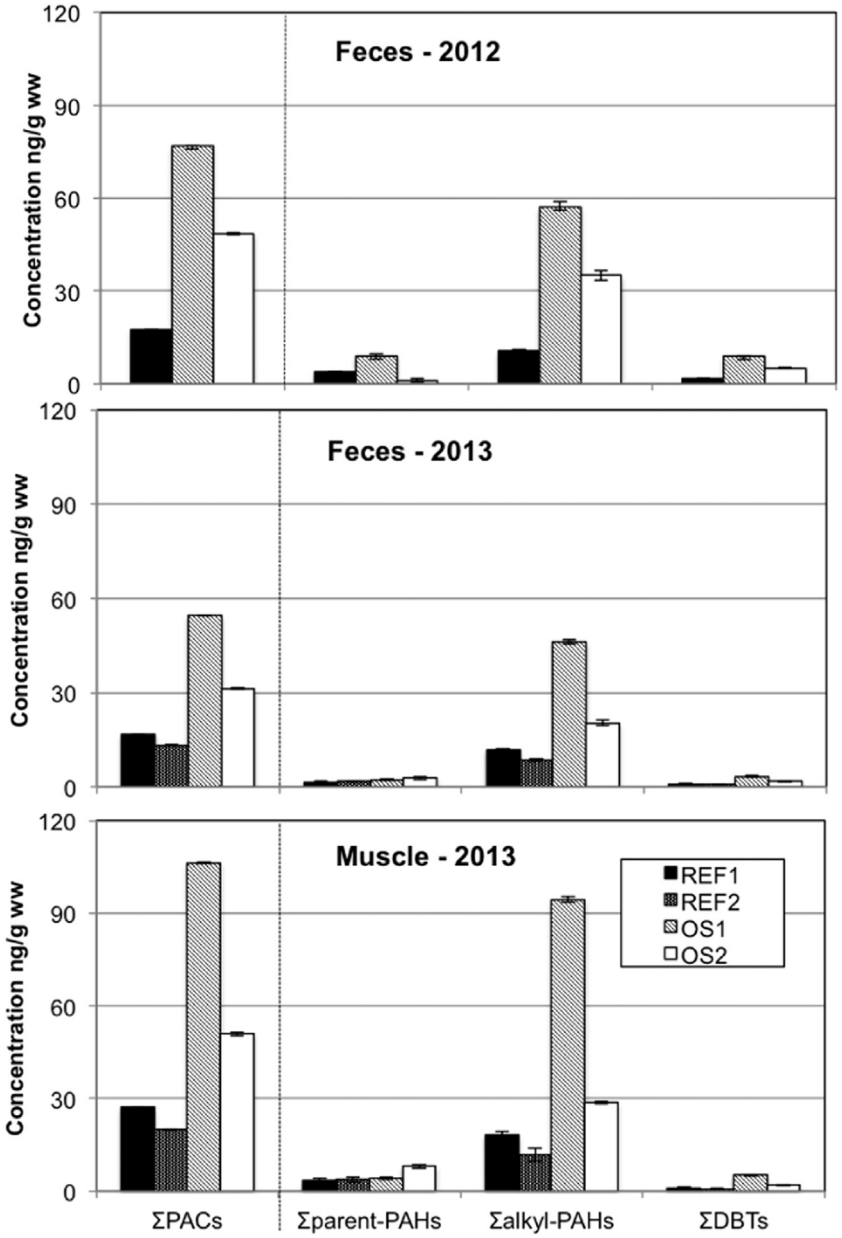

Fig. 2. Concentrations of ΣPACs, Σalkyl-PAHs, and ΣDBTs in nestling tree swallows were similar in the reference birds (REF1, REF2), and significantly higher in the birds at the Oil Sands mining-related (OS1, OS2) sites in the Athabasca Oil Sands Region (*p-values* ≤ 0.03).

3.3. Stable isotopes: proxies of diet

In 2013, the $\delta^{15}N$ values of the chicks significantly differed among the sites ($X^2 = 15.33$, $p = 0.002$), with $\delta^{15}N$ values similar among the reference birds (REF1: 7.7 ± 0.2; REF2: 7.8 ± 0.03) and higher than the birds at OS1 (6.9 ± 0.1) and OS2 (7.2 ± 0.1). The $\delta^{13}C$ values also significantly differed among the four sites (main effect site: $F_{4,69} = 22.17$, $p < 0.001$) in relation to the season progressing (Julian lay date: $F_{1,69} = 42.73$, $p < 0.001$); $\delta^{13}C$ values were similar in birds at REF1 (−30.2 ± 0.2) and OS1 (−29.4 ± 0.5), but comparatively higher in chicks at REF2 (−25.5 ± 0.4) and OS2 (−27.5 ± 0.4).

3.4. PACs and environmental matrices

Compared to the reference birds, the nestlings at OS1 and OS2 were exposed to higher air concentrations of ΣPACs (3–8× higher), Σparent-PAHs (2–8× greater), Σalkyl-PAHs (4–7× greater), ΣDBTs (9–41× higher), and the major PAHs (Figs. 6, 7). Similarly, OS2 had considerably higher water concentrations of ΣPACs (4–5×), Σparent-PAHs (2–3×), Σalkyl-PAHs (4–5×), and the majority of major PAHs, than the other three study sites (Figs. 6, 7). Water concentrations of ΣDBTs were 4 to 9× higher at OS1 and OS2 compared to the reference sites, but the reference sites had higher water concentrations of C1, C2-naphthalenes, and C4-fluoranthenes/pyrene.

3.5. Sources of exposure to PAHs for nestling tree swallows

In terms of the muscle concentrations of the major PAHs, stable isotope measures (as proxies of diet) were identified as important variables in the best models for C1-naphthalenes, C2-naphthalenes, and C2-fluorenes (w_is = 0.50–1.00), suggesting that the birds' diet may be the most important source of exposure to these particular PAHs. Individually, $\delta^{15}N$ accounted for 7%–28% of the variation in muscle concentrations of C1– and C2-naphthalenes, and C1-fluorenes. There was minimal influence of $\delta^{13}C$, accounting for only 2% of the

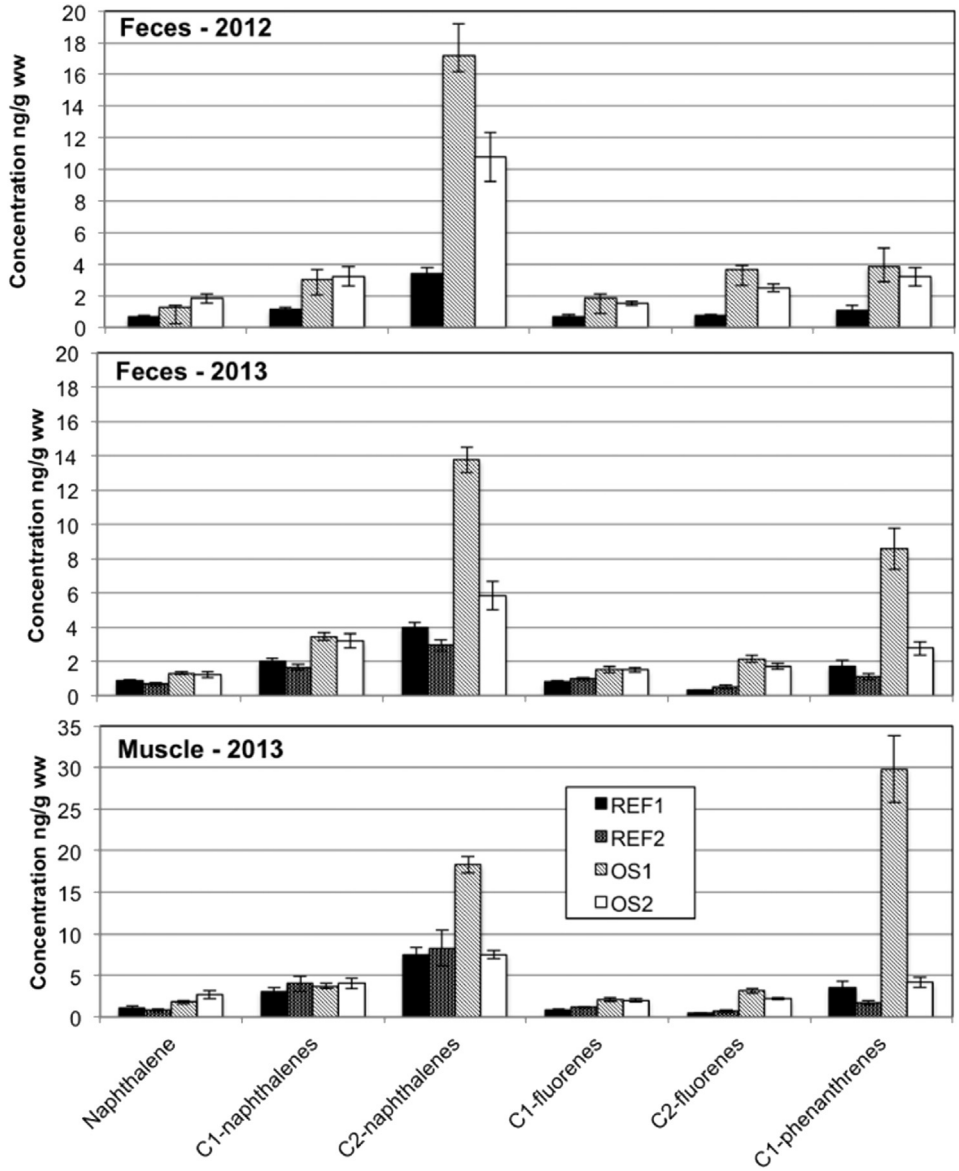

Fig. 3. The concentrations of the six major PAHs measured in nestling tree swallows were similar between the reference birds (REF1, REF2), and significantly higher in the birds from the Oil Sands mining-related sites (OS1, OS2) in the Athabasca Oil Sands Region (*p-values* ≤ 0.05).

variation in muscle C2-fluorene concentrations. Dietary $\delta^{13}C$ and $\delta^{15}N$ accounted for 16% of the variation in C1-naphthalenes (Table S1). Air concentrations significantly explained some variation in the birds' muscle concentrations of C2-naphthalenes and C1-fluorenes, whereas water concentrations explained some variation in muscle C1-phenanthrenes, suggesting that air and water may be important sources of exposure to these individual PAHs for birds (Table S1). Hatch date (C2-naphthalenes, C2-phenanthrenes) and nestling sex (C1-fluorenes) had some influence on muscle concentrations of these major PAHs, with no influence of regional ambient temperatures or precipitation during the nestling period. Muscle naphthalene concentrations were unrelated to any of the measured fixed factors.

For fecal PAH concentrations, limited variation in the three of the 6 major PAHs was explained by the fixed factors measured in this study. Fecal C1-phenanthrenes were explained by water concentrations of the same PAH (40%) and minimally by nestling body mass (4%) (Table S1). Fecal C2-fluorenes were best explained by air concentrations and nestling sex ($w_i = 0.89$), explaining 12% of the variation. The best model for C1-naphthalenes was $\delta^{13}C$ which explained 6% of the variation. Statistical details are provided in Tables S1 and S2.

4. Discussion

Previous research using tree swallows nesting in the Athabasca Oil Sands Region have provided an understanding of avian exposure to metals and metalloids (Godwin et al., 2016), air contaminants (Cruz-Martinez et al., 2015), naphthenic acids (Gentes, Waldner, 2007), and oil-sands processed affected material (OSPM) (Gentes et al., 2006). However, to date, little research has characterized the PAH exposure of regional wildlife relative to OS mining-related activities. Prior to the present study, only two studies have previously characterized PAC concentrations in vertebrates in this region, specifically in fish and mammals (Lundin et al., 2015; Ohiozebau et al., 2016). Endocrine changes in nestling tree swallows were hypothesized to be related to mining-associated chemicals including PAHs, and environmental factors (Gentes, McNabb et al., 2007), while the nestling swallows in the current study experienced increased hepatic detoxification (measured by EROD induction) when exposed to various air contaminants (Cruz-Martinez et al., 2015). Exposure to alkyl-PAHs and heavy metals were correlated with poorer body condition, smaller testes, and/or oxidative stress in laboratory mice that were surrogates for deer mice

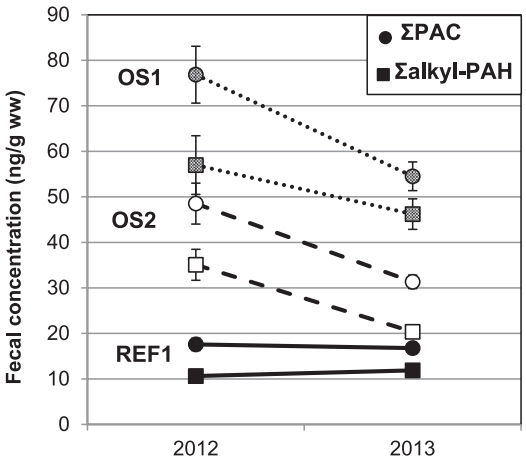

Fig. 4. The concentrations of ΣPACs, Σalkyl-PAHs, and ΣDBTs (not shown) in feces of nestling tree swallows were significantly higher in 2012 than 2013 at the two Oil Sands mining-related sites (OS1, OS2) but similar between years at the reference site (REF1) in the Athabasca Oil Sands Region. REF2 was established in 2013, not 2012, and thus was excluded from this analysis.

(*Peromyscus maniculatus*) inhabiting a reclaimed mine site in the OS Region (Rodríguez-Estival et al., 2015). Our study is the first to characterize the exposure, uptake, and sequestering of PACs by wild birds in the Athabasca Oil Sands Region.

The present study identifies that tree swallow nestlings inhabiting the Athabasca OS Region are exposed to and sequester a complex mixture of PACs, with increased exposure and uptake evident in the tree swallows nesting within 5 km of active mining industrial sites in the region. Consistent with previous findings in which PAH concentrations in the stomach contents of nestling tree swallows were significantly correlated with sediment concentrations in the Great Lakes (Custer et al., 2017), our current findings suggest that diet, indicated particularly by $\delta^{15}N$ more than $\delta^{13}C$, is a major route of exposure for some measured PACs, with air and water at the breeding colonies serving as additional important sources of exposure to PACs for these birds. All 21 analyzed parent PAHs, all 13 alkyl-PAHs, and 4 out of 5 DBTs, were detected in air, water, and nestling tree swallows (feces, muscle), with most higher at the OS sites than the reference sites. PAC concentrations in the environmental matrices and nestlings were significantly higher in 2012 than 2013 possibly because of variable mining-related activity and/or weather between years. Despite the 100-year record rains that occurred during the nestling period in 2013, there was no effect of regional weather on nestling PAC burdens.

4.1. PACs in birds

The results of the present study indicate that birds in the region are exposed to and accumulate methylated DBTs, various parent PAHs (i.e., benzo[*e*]pyrene, benzo[*k*,*i*]fluoranthene, benzoflurenes, retene, biphenyl, phenyltoluenes, dibenzofuran), and several alkyl-PAHs (i.e., C1-benzo[*a*]pyrenes, C2-, C3-fluorenes, C1-fluoranthenes/pyrene, C1-benzo[*a*]anthracenes). Our results are also consistent with two previous studies that reported increased exposure to PACs (Lundin et al., 2015; Ohiozebau et al., 2016), and the dominance of alkyl-PAHs relative to parent PAHs (Lundin et al., 2015), in fish and mammals inhabiting areas closer to mining activities (Lundin et al., 2015; Ohiozebau et al., 2016). In 2010–11, mean ΣPAH levels were much higher (48 ng/g wet mass (wm)) in fish from the Athabasca River closest to mining activity, than fish downstream near Lake Athabasca (13 ng/g wm) (Ohiozebau et al., 2016). The ΣPAH concentrations in the fish are similar to those in the present tree swallows, despite obvious differences in habitat use, and show the same pattern of higher concentrations at OS sites (feces: 31.3–76.8 ng/g ww; muscle: 50.9–106.3 ng/g ww) than at remote reference sites (feces: 13.3–17.7 ng/g ww; muscle: 19.9–27.1 ng/g ww). Like the tree swallows, fecal samples from moose and wolves in the actively mined area had higher alkyl-PAHs relative to parent PAHs (Lundin et al., 2015).

4.2. PACs in the abiotic environment

Air and water are two possible routes of exposure to PAHs and other contaminants for wildlife. Previous research has focused on the broad suite of contaminants released into the environment by regional mining activities (Kelly et al., 2009; Brown and Ulrich, 2015; Huang et al., 2016), but only a few have reported specifically on PACs in the region. Regional air concentrations of parent PAHs are comparable to those in industrial and urban sites in Europe and North America, with similar concentrations of lower molecular weight alkyl-PAHs as found in urban sites, and a greater abundance of higher molecular weight alkyl-PAHs than elsewhere (Schuster et al., 2015). Aerial ΣPAC concentrations measured in the current study were either similar (2012) or higher (2013) than those previously reported in the OS Region (2010—2012), with a similar geographical pattern of higher concentrations of Σparent PAHs, Σalkyl-PAHs, and ΣDBTs at OS sites than at more remote reference sites (Schuster et al., 2015). Our findings also reflect previous research

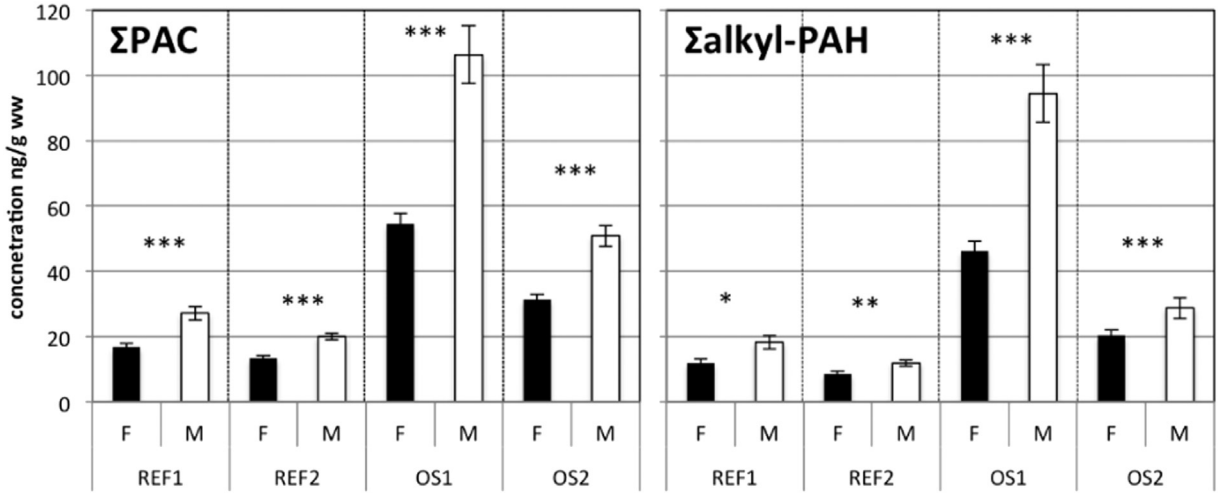

Fig. 5. In 2013, concentrations of ΣPACs, Σalkyl-PAHs, and major PAHs (data not shown) were significantly higher in muscle (M) than feces (F) in nestling tree swallows from reference sites (REF1, REF2) and Oil Sands sites (OS1, OS2) in the Canadian Oil Sands (Alberta) ($t < 4.89$; *p-values* < 0.02). ***$p < 0.0001$; **$p < 0.01$; *$p \leq 0.05$.

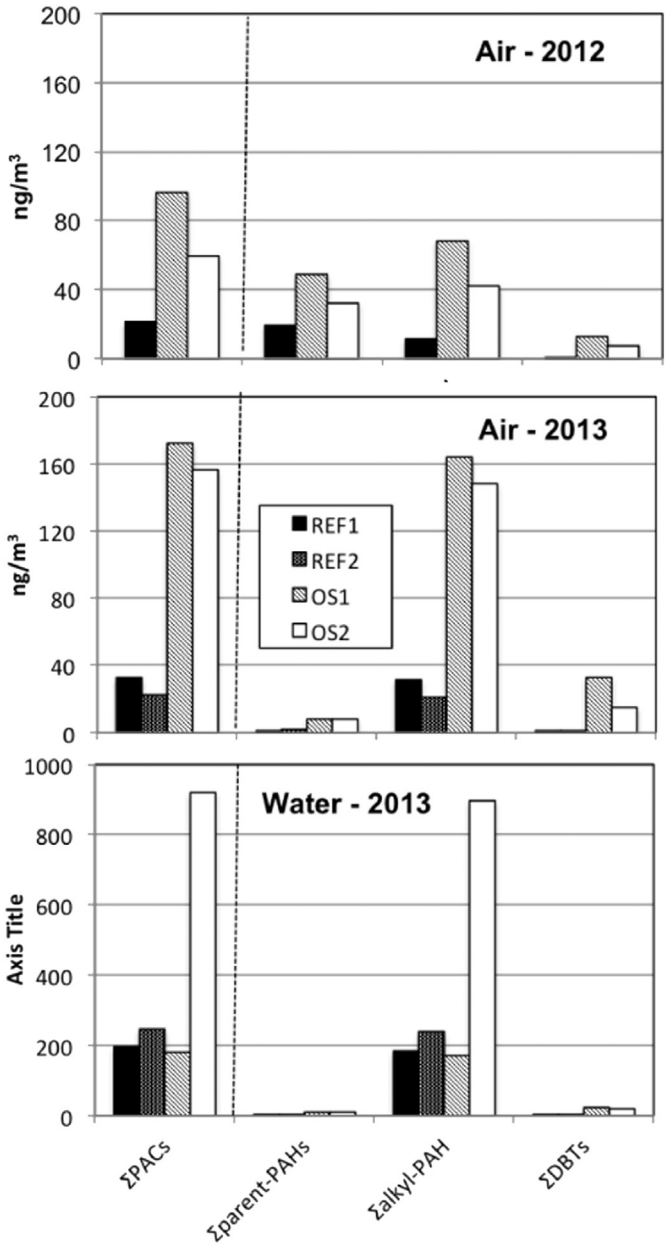

Fig. 6. Concentrations of ΣPACs, parent PAHs, Σalkyl-PAHs and ΣDBTs in air and water from two reference (REF1, REF2) and two mining-related (OS1, OS2) sites in the Athabasca Oil Sands Region (Alberta, Canada). Naphthalene was not detected in the passive air samplers in 2013, and as a result, has been excluded when comparing concentrations of Σparent-PAHs between years.

using passive air samplers in the region, with a dominance of alkyl-PAHs and the presence of ΣDBTs, and decreasing concentrations of ΣPAHs, Σalkyl-PAHs and ΣDBTs with increasing distance from active mining (Schuster et al., 2015; Jariyasopit et al., 2016); thus the same pattern as observed with the PAC concentrations measured in our tree swallow nestlings.

A comparable, wide array of PACs were detected previously in sediments and core samples, with a similar domination of alkyl-PAHs in recent sediment (Wayland et al., 2008; Elms et al., 2016; Korosi et al., 2016; Jautzy et al., 2013) as we found in the water adjacent to the nest boxes at all four study sites. In a previous study at reclaimed wetlands directly receiving OSPW, there were higher concentrations of alkylated naphthalenes, fluorenes, phenanthrenes/anthracenes and fluoranthrenes/pyrenes (combined), than parent PAHs or alkylated DBTs, in sediments and insect larvae (Wayland et al., 2008). In the current study, sites were selected to avoid direct input of OSPW into the waterways, suggesting that the PAHs measured in the water are likely resulting mainly from atmospheric deposition, as well as the seams of oil-impregnated sand naturally occurring in the region. Aerial deposition of PACs was previously isolated in snowpack that accumulated more volatile forms and 5-ring PAHs by atmospheric transfer and was dominated by phenanthrenes, fluorenes and dibenzothiophenes (Kelly et al., 2009).

In the present study, the PAC profiles varied to some degree between air and water. Of the parent PAHs, naphthalene especially, and phenanthrene, had the highest concentrations in air and water, particularly at the two OS sites. Concentrations of the other parent PAHs were similar across sites. Of the alkyl-PAHs, generally the alkylated naphthalenes and fluorenes dominated both environmental matrices, particularly in 2013, but otherwise, alkyl-PAH patterns were inconsistent between the environmental matrices among the four study sites, likely reflecting various sources of PACs to the water bodies as discussed above.

4.3. Routes of exposure to PAHs for birds in the OS region

Wildlife may be exposed to a mixture of chemicals through diet, water (drinking, swimming), inhalation, preening of feathers, dermal exposure, and/or direct ingestion of sediments; the latter was linked to mortality of water birds in mining areas (Beyer et al., 2000; Hoffman et al., 2000). Consequently, attributing the relative importance of these exposure pathways is complex. In the OS region, higher concentrations of PACs may be found in water and air nearer to mining sites (reviewed above) as confirmed in the present study. Aquatic-emerging aerial insects, typically an important food source for tree swallows (Winkler et al., 2011) including in the OS Region (Godwin et al., 2016), accumulate PACs (Custer et al., 2017) regardless of OSPW-inputs to waterways (Wayland et al., 2008). Custer and colleagues (Custer et al., 2017) demonstrated that PAH concentrations were correlated between sediment and stomach contents of nestling tree swallows. Thus, tree swallows may receive exposure to PACs from their diet, from drinking or bathing in water, and/or from air by inhalation or preening of their feathers through contaminants sticking to preen oil (Jaspers et al., 2004; Jaspers et al., 2007). Determining which environmental compartments may be contributing to the PAC body burden of terrestrial-based wildlife in the OS Region would be useful for future monitoring and remediation efforts. Here, we identified that there were multiple, differing routes of exposure to each PAC congener for regional tree swallows.

Dietary exposure to contaminants is often the most influential exposure route affecting body burdens (Rozman and Klaassen, 2003). The variation in most of the major PAHs measured in the tree swallow nestlings was partially explained by stable isotopes, suggesting that diet is one of the key factors involved in the exposure and uptake of PACs by these birds. This is consistent with the positive correlation found between $\delta^{15}N$ and total mercury in colonial water bird eggs collected across a larger area that included the Oil Sands Region (Hebert et al., 2011), and with the conclusion by Farwell and colleagues (Farwell et al., 2014), that $\delta^{15}N$ may be especially useful for tracing dietary exposure of tree swallows to OSPM elements in this area. In the current study, the influence of $\delta^{15}N$ and $\delta^{13}C$ varied greatly by congener and by matrix. Muscle $\delta^{15}N$ alone explained 20–28% of the variation in concentrations of C2-naphthalenes and C1-fluorenes, and was negatively correlated with muscle PAHs and positively related to fecal C2-naphthalenes. While typically $\delta^{15}N$ increases with trophic position (reviewed in (Kelly, 2000)) and concentrations of contaminants, signatures of $\delta^{15}N$ can be affected by environmental variables such as temperature and nutritional status (reviewed in (Jardine et al., 2006)). Moreover, tree swallows in the OS region consume both terrestrial and aquatic-emerging insects (Godwin et al., 2016). Our results of the relationships with muscle $\delta^{15}N$ and PAC concentrations demonstrate

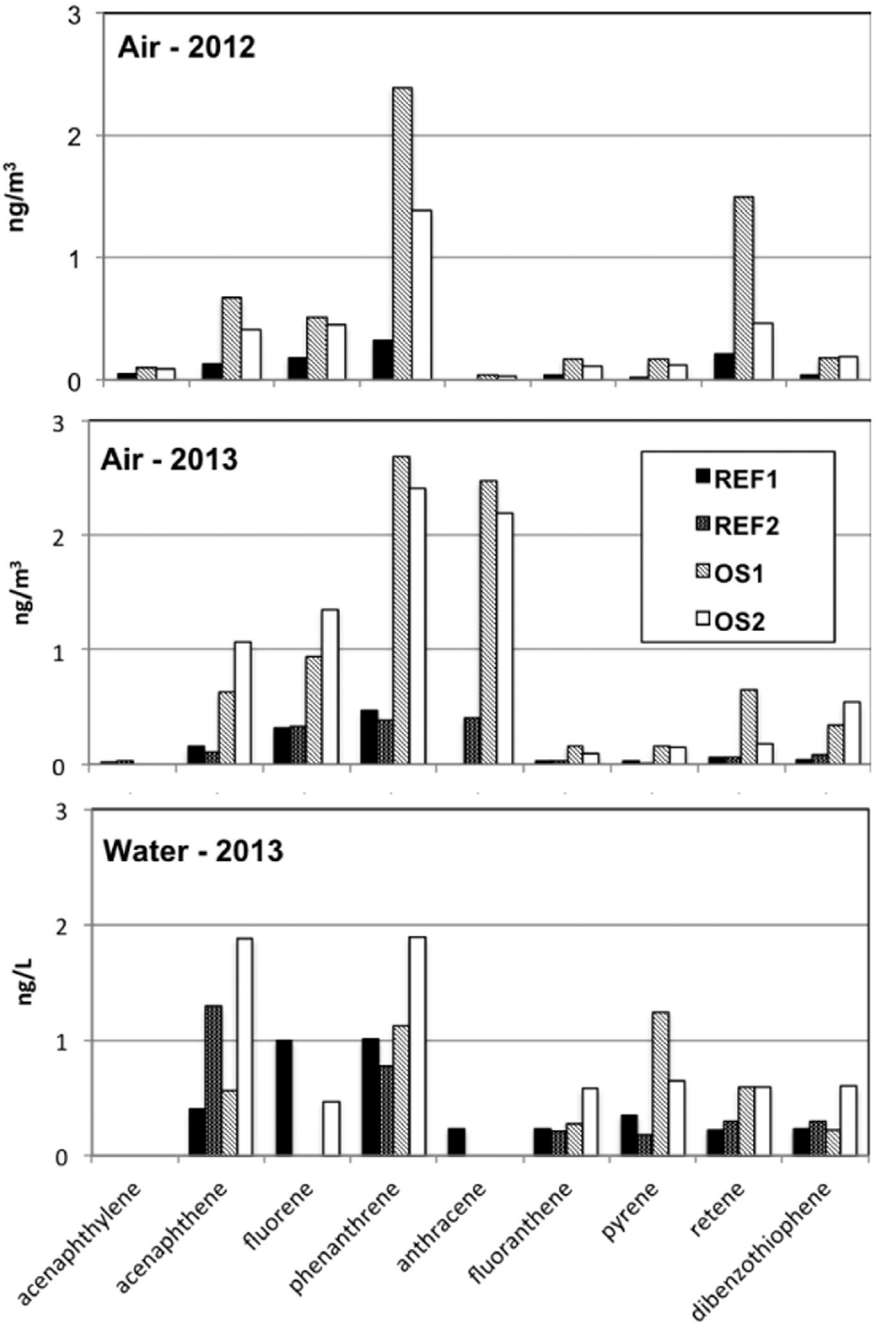

Fig. 7. Concentrations of parent PAHs (except naphthalene) in the air and water adjacent to colonies of breeding tree swallows at reference (REF1, REF2) and Oil Sands mining-related sites (OS1, OS2) in the Athabasca Oil Sands Region.

that the composition of the birds' diet is related to the PAC concentrations in their tissues.

Tree swallows eat a wide variety of emerging insects (Mengelkoch et al., 2004) that may have varying PAH concentrations in the Oil Sands Region (Wayland et al., 2008). PAH concentrations were higher in samples that included mainly chironomids (suborder: *Derotanypus*), damselflies (suborder: *Zygoptera*), and caddisflies (genus: *Phryganea*), compared to dragonflies (suborder: *Anisoptera*) (Wayland et al., 2008). Differences in PAH concentrations in these insect groups may be related to differences in their body size, freshwater habitats used, feeding habits, trophic position, their ability to metabolize PAHs, and whether or not they have moulted their exoskeletons that contain much of the accumulated contaminants ((Wayland et al., 2008) and references therein). Different groups of insects, including some of the same groups, also show significantly different $\delta^{15}N$ signatures (Bennet and Hobson, 2009); this may partially explain the differences in the stable isotope signatures of the tree swallow nestlings across our study sites, and hence differences in their exposure and accumulation of PACs. Further research on trophic transfer of PAHs in this ecosystem would be beneficial to determine how variations in diet affect avian body burdens of PACs in the Oil Sands Region.

Dietary sources are indicated by $\delta^{13}C$ (Kelly, 2000) and are not expected to vary greatly among tree swallows that feed in freshwater-terrestrial habitats (reviewed in: (Kelly, 2000)). As expected, our results suggest that $\delta^{13}C$ had minimal influence on the birds' PAC concentrations, since $\delta^{13}C$ alone only explained 2% of the variation in muscle C2-fluorenes. As well, $\delta^{13}C$ explained only very small amounts of variation in fecal C1-naphthalenes (6%) and C2-fluorenes (8%). Together with $\delta^{15}N$, $\delta^{13}C$ explained 16% of the variation in muscle C1-naphthalenes. Muscle concentrations of naphthalene and C1-

phenanthrenes were not influenced by either of these two stable isotopes, suggesting that diet may not be the main route of exposure for the tree swallows to these particular contaminants.

While the freshwater bodies in our study areas likely receive mostly aerial deposition of PACs (and not OSPW), the slightly different PAC concentrations and profiles in the air and water at our sites may have contributed differentially to the PAC body burdens of the swallows. We determined that of the 6 major PAHs, the variation of three low molecular weight alkyl-PAHs was influenced by air concentrations, including two PAHs with the highest air concentrations in 2013: C1-fluorenes in muscle and C2-fluorenes in feces, plus C2-naphthalenes in feces. Air concentrations alone accounted for 33% of the variation in muscle C1-fluorenes but only 9% of fecal C2-fluorenes, and in combination with $\delta^{15}N$ accounted for 35% of the variation in C2-naphthalenes. These results demonstrate the importance of air-deposition exposure pathways in tree swallows taking up and accumulating these compounds. Only the C1-phenanthrenes in the birds were influenced by water concentrations of C1-phenanthrenes, accounting for approximately 40% of the variation in either fecal or muscle concentrations. Since the dietary stable isotopes did not explain any variation in the fecal or muscle concentrations of C1– phenanthrenes, our results suggest that water concentrations may be a more important source of exposure than demonstrated here, warranting further research.

4.4. Monitoring and research of PAHs and birds

In terms of long-term monitoring of PACs, our results support the use of fecal samples from nestling tree swallows as a non-lethal, easily employed sampling methodology for research and monitoring. While fecal PAC concentrations were lower than muscle concentrations of PACs, they were highly correlated. However, the present results suggest that fecal PAC concentrations are less reflective of environmental or potential dietary PAC concentrations, with only a minimal amount of variation explained in the fecal concentrations of three of the 6 major PACs (6–12%). These results suggest that fecal sampling of birds characterizes their overall exposure to PACs but not the sources of exposure to PACs for the birds, while analyzing their tissues (e.g., muscle) identifies the potential and various sources of PAC exposure, and the accumulation and sequestering of PACs by the birds. Based on these results, we suggest that future research and monitoring of regional birds in relation to PACs should involve fecal collections (e.g., annually) to identify and monitor avian exposure to PACs. When necessary, non-lethal collections would be conducted in conjunction with lethal sampling of birds for tissue collections, to assist in determining potential environmental sources of avian exposure to PACs; stable isotopes or another method to identify diet, and sample collections of environmental matrices (e.g., sediment, water, air), would also be required with the lethal sampling to identify potential environmental sources of PACs for the birds.

5. Conclusion

The Athabasca Oil Sands Region has been intensively mined and recent studies have demonstrated an increase in a complex mixture of PAC concentrations in the environment (Kurek et al., 2013). Until the completion of this study, there was little information available regarding the exposure, uptake and deposition of PACs by wildlife, particularly birds, living in close proximity to mining activities. To the best of our knowledge, we have presented the most comprehensive study to date identifying the exposure and uptake of PACs by vertebrates in the OS Region, and the first describing PAC tissue concentrations of birds in relation to concentrations in the air, water, and their diet. Tree swallows that bred in close proximity to Oil Sands mining sites were exposed to higher air and water concentrations of a complex mixture of 41 of 42 PACs, dominated by alkyl-PAHs plus parent-PAHs and DBTs. Nestlings at Oil Sands mining-related sites accumulated higher concentrations of PACs (muscle, feces) than the reference chicks through varying routes of exposure. Diet was likely the most important route of exposure to some of the major PACs (C1-naphthalenes, C2-naphthalenes, C1-fluorenes) whereas air (C1-fluorenes) or water were sources of other PACs (C1-phenanthrenes). Research on the toxicity to birds of this complex mixture of PACs is a critical next step in assessing potential risks to wildlife inhabiting the Athabasca Oil Sands Region.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2017.12.123.

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