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Reproductive and developmental changes in tree swallows (*Tachycineta bicolor*) are influenced by multiple stressors, including polycyclic aromatic compounds, in the Athabasca Oil Sands☆

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ABSTRACT

Mining in the Athabasca Oil Sands Region (AOSR) has contributed extensively to increased exposure of wildlife to naturally occurring polycyclic aromatic compounds (PACs), yet little is known about the toxicity of PACs to wildlife in this region. We identified reproductive and developmental changes in tree swallows (*Tachycineta bicolor*) breeding in close proximity to mining-related activities in the AOSR, and determined these changes in relation to the birds' exposure and accumulation of 41 PACs (parent-, alkylated-PAHs), dibenzothiophenes (DBTs; previously published), diet (carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$)), volatile organic compounds, and weather variables. Tree swallow pairs ($N = 43$) were compared among mining-related (OS1, OS2) and reference (REF1, REF2) sites. At OS2, clutch initiation was slightly advanced (2012) but reproductive success (65%) was much lower than at the other sites ($\geq$ 79%). Fledgling production by each pair was influenced by the timing of clutch initiation (years combined); in a highly inclement brood rearing period (2013), additional influences included the nestlings' exposure to ΣDBTs, accumulation of C1-naphthalene, the trophic position of the prey in their diet ($\delta^{15}N$), and record-breaking rainfall. Nestlings at OS2 were significantly lighter at day (d) 9 and d14, and in poorer body condition (d9). Nestling body mass was influenced by multiple stressors that varied by site: mass of younger nestlings (d9) was related to dietary source ($\delta^{13}C$; e.g., wetlands, terrestrial fields), exposure and/or accumulation of C1-phenanthrenes, C2-fluorenes, Σalkyl-PAHs and ΣDBTs, while for older nestlings (d14), body mass was related to sex, hatch date and/or rainfall during brood rearing. The swallows' exposure and accumulation of parent-PACs, alkyl-PACs and DBTs, the timing of hatching, their diet and exposure to highly inclement rains, contributed to their reproductive and developmental changes.

1. Introduction

The Athabasca Oil Sands Region (AOSR) in northern Alberta and Saskatchewan, Canada, is the third largest known oil reserve in the world (Government of Alberta, 2012), and has been actively mined since the 1960s (CCAoP, 2016a). In 2015, 3.98 million barrels of crude oil were extracted daily and this is projected to increase to 5.45 million barrels by 2030 (Giesy et al., 2010; Parajulee and Wania, 2013; CCAoP, 2016b). Polycyclic aromatic compounds (PACs) occur naturally in the region but are also introduced by mining activities through the seepage of oil sands processed waters (OSPW) into local waterways and by aerial emission and deposition (Kelly et al., 2009; Zhang et al., 2016). Since industrial extraction began, there has been a significant increase (2.5–23-fold) in the concentrations of many PACs in lake sediment cores, suggesting a

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direct effect of this mining activity on the local environment (Kurek et al., 2013).

Few studies to date, have characterized the exposure of wildlife to PACs in the AOSR. Tree swallows (*Tachycineta bicolor*) are a small migratory passerine that consume aquatic-emerging insects and are known to accumulate numerous contaminants (e.g., 9). In the AOSR, tree swallows are exposed to and accumulate a wide array of PACs, including parent polycyclic aromatic hydrocarbons (PAHs), alkylated PAHs (alkyl-PAHs) and dibenzothiophenes (DBTs) through their diet, air (inhalation, preening of feathers), and from on-site fresh water bodies that do not directly receive OSPW (Fernie and Marteinson et al., 2018). Concentrations of 41 PACs were elevated in nestling tree swallows (31–106 ng/g wet weight (ww)) raised near mining-related activities compared to nestlings at reference sites (13–27 ng/g ww) (Fernie and Marteinson et al., 2018). Parent PAHs have also been described in fish (Ohiozebau et al., 2016) and a range of PACs have been detected in feces of large mammals (Lundin et al., 2015) inhabiting the AOSR. The PAC profiles in both mammals (Lundin et al., 2015) and tree swallows (Fernie and Marteinson et al., 2018) were dominated by alkylated PAHs and included DBTs, suggesting petrogenic origins associated with recent mining activity (Schuster et al., 2015 and references therein).

Most research concerning the potential effects of PACs has focused on parent PAHs, a group of PAHs that are not dominant in biota in the AOSR. Previous studies have reported that the exposure of birds to parent PAHs elicits a range of physiological, reproductive and developmental effects including changes in egg production, reduced hatching success, and increased abandonment of nestlings (reviewed in Albers, 2006; Leighton, 1993). Moreover, embryonic exposure of birds to parent PAHs also elicits various physiological effects in embryos and nestlings, reduces their growth, increases the incidence of deformities, and can cause acute toxicity (reviewed in Albers, 2006; Leighton, 1993). The effects and risks of exposure to other types of PACs (e.g., alkyl-PAHs) is largely unknown although some alkyl-PAHs have mutagenic effects and may be partially responsible for the toxic effects of environmental mixtures of PAHs (reviewed in Baird et al., 2007). Laboratory mice were exposed to three alkyl-PAHs at concentrations likely experienced by wild mice on reclaimed mine sites in the AOSR, and exhibited subclinical toxicological effects including changes in relative organ size (liver, kidney, spleen), suppressed homeostasis of hepatic vitamins A and E, and altered testicular oxidative status suggesting the potential for reproductive changes (Rodriguez-Estival et al., 2015).

In terms of the complex mixtures of PACs found in the AOSR, only a few studies have examined the toxic effects, if any, on the physiology and reproduction of wildlife inhabiting the AOSR. On a reclaimed mine site in the AOSR, reproducing deer mice (*Peromyscus maniscculatus*) were exposed to elevated metals and likely PACs, and were found to be in poorer condition, with smaller testes and increased testicular and hepatic oxidative stress, hypothesized to affect testicular function (Rodriguez-Estival et al., 2015, 2016). Reproduction of fish, both their spawning rate and the number of eggs produced per female, was reduced when they were exposed in the laboratory to OSPW containing high concentrations of naphthenic acids (Kavanagh et al., 2011). Hatching success and fledging success were inconsistently reduced across years for tree swallows that bred at one of several reclaimed wetlands in the AOSR where sediments and water were contaminated with naphthenic acids, parent- and alkyl-PAHs, and DBTs (Smits et al., 2000). In a previous study, the mortality rate of nestling tree swallows was greater for those birds on some reclaimed wetlands when they experienced extreme inclement weather with extreme rainfall and rapidly declining temperatures (by 16 °C) within 24 h; the authors concluded these birds were less fit to survive these multiple stressors (Gentes et al., 2006). The two studies (Smits et al., 2000; Gentes et al., 2006) did not examine if the reproductive changes were associated with the birds' exposure to PACs, representing an important knowledge gap. Furthermore, no studies to date have evaluated whether avian reproduction is altered on sites within the AOSR that do not receive OSPW directly.

Using tree swallows as an avian model, the major objectives of the present study were to investigate and characterize reproductive and developmental changes in birds in relation to their exposure and accumulation of parent- and alkyl-PAHs and DBTs in the AOSR (Fernie and Marteinson et al., 2018). We compared birds breeding near OS mining-related activities at sites not directly receiving OSPW, with tree swallows breeding at reference sites. We also sought to determine if any reproductive and/or developmental changes observed in the tree swallows were related to factors known to influence these parameters in birds in general, specifically the diet of the nestlings using stable isotopes as dietary proxies, air quality measures, and weather variables since an extreme weather event that broke 100-year rainfall records in the region occurred in 2013 during the course of this study.

2. Materials and methods

2.1. Study sites and subjects

The conduct of this study, including all related protocols involved in handling and monitoring of the tree swallows, was approved in accordance with the guidelines of the Canadian Council of Animal Care. Appropriate permits were obtained from the Canadian Wildlife Service and all provincial (Alberta) agencies. As previously described (Fernie and Marteinson et al., 2018; Cruz-Martinez et al., 2015), nest boxes (N = 15–34 per site) for tree swallows were established ~3 m apart within 10–20 m of on-site fresh water sources, at two study sites (OS1, OS2) within 5 km of active mining (mine pits, processing plants), and at two reference sites (REF1, REF2) ~60 km south of Fort McMurray in the AOSR (Fig. 1). At each study site, the freshwater bodies did not receive OSPW directly. With the exception of REF2 that was established in 2013, tree swallow nests were monitored for two years (2012, 2013) at the other three study sites.

Adult tree swallows began to occupy nest boxes between May 10th and 20th and were monitored daily until clutch completion (no further eggs were laid for 3 days (d)). Adults were not captured because of logistical constraints, and hence were not aged or banded. During incubation, nests were left undisturbed for the initial 9–10 d. Thereafter they were again monitored daily and hatch dates recorded. Morphometrics (body weight, wing chord and ninth primary feather length) were recorded for each chick on d 9 and d 14. The body weight:wing chord ratio was calculated for each nestling as an estimate of the condition index of each individual (Labocha and Hayes, 2012). Chicks were assessed at 14 d of age in order to avoid premature fledging and abandonment, and were considered to have fledged at this time. At that time, two or three nestlings per brood were randomly selected and a volunteered fecal sample from each individual was collected directly into a cryovial, flash frozen in liquid nitrogen and stored at −80 °C until chemical analysis. As previously described (Cruz-Martinez et al., 2015), each of the selected nestlings was anesthetized in an enclosed, vegetation-lined chamber which contained an isofluorane-soaked cotton ball, followed by cervical dislocation, and then dissection. Nestlings were sexed and examined for deformities and obvious physical signs of disease. A 1 g sample of pectoral muscle was stored in a cryovial in liquid nitrogen, flash frozen and stored at −80 °C until chemical analysis. As previously described (Fernie and Marteinson et al., 2018), dorsal feathers were

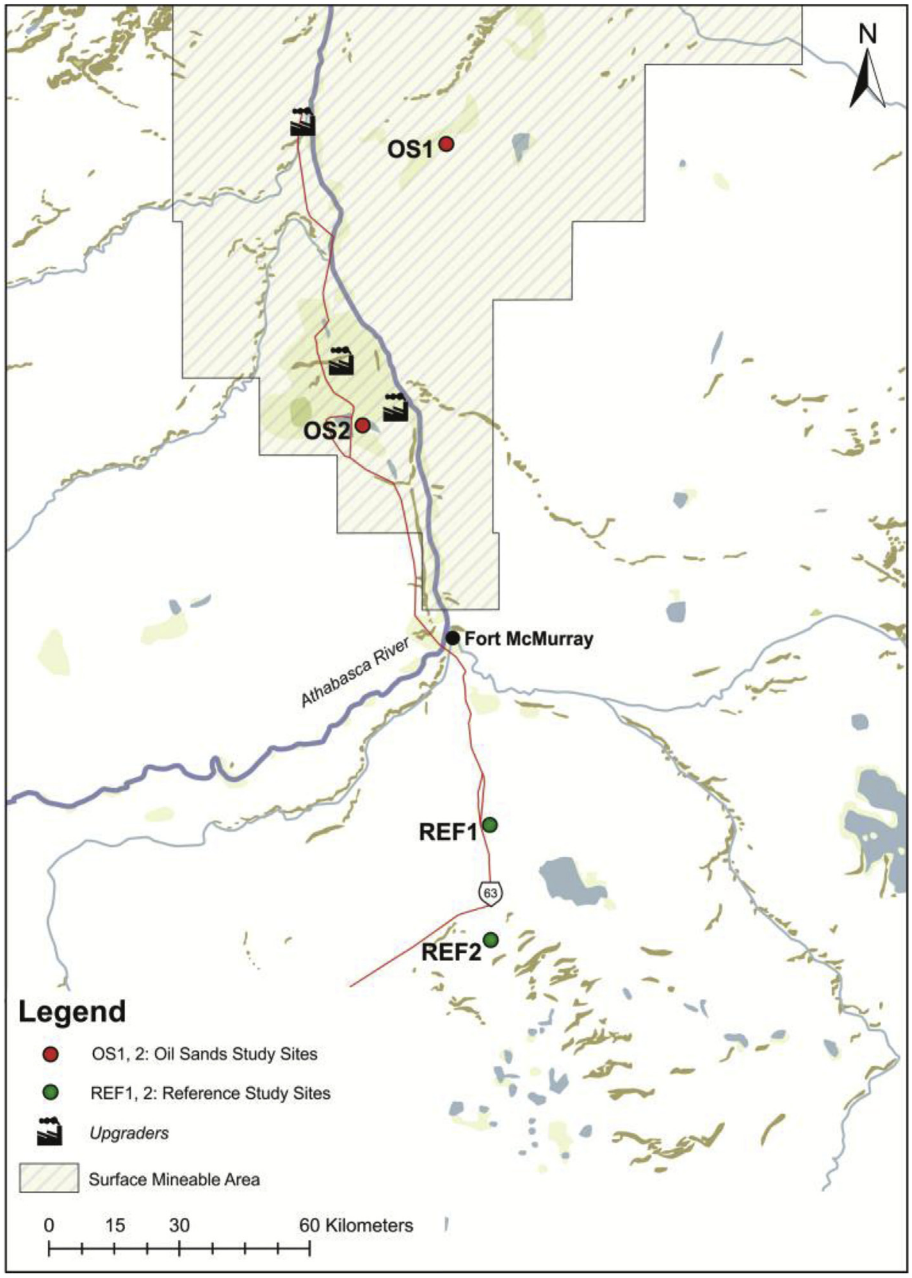

Fig. 1. The study sites where tree swallows bred in nest boxes and were monitored included two reference sites (REF1, REF2) and two oil sands mining-related sites in the Athabasca Oil Sands Region of Alberta, Canada. Previously published in Fernie and Marteinson et al. (2018).

collected for stable-isotope analysis ($\delta^{15}C$ and $\delta^{13}N$).

For each pair of adult tree swallows, we recorded the Julian dates of the first and last eggs being laid, when incubation began, and the hatching date of the first nestling. The duration of egg-laying (number of days between the first and last egg laid) and incubation (day after last egg was laid until the first egg hatched) were also calculated for each pair, as was clutch size (the number of eggs laid per pair), egg laying patterns (eggs laid/d), hatchling and fledgling production representing the numbers of hatchlings and fledglings produced by each pair, hatching success (the percentage of eggs within a clutch that hatched), fledging success (the percentage of hatchlings within a brood that fledged), and overall reproductive success (the percentage of eggs within a clutch that fledged).

2.2. Weather and volatile organic compounds

Using data collected by Environment and Climate Change Canada (ECCC) and Wood Buffalo Environmental Association (WBEA), we calculated the means of weather (ambient temperatures, precipitation) and air quality variables specific to each pair of swallows for the 5 d before they began to lay their clutch of eggs and their 14 d brood rearing period. Total precipitation (mm), the number of rainy (>1 mm/d) or heavy rain days (>10 mm/d), and regional precipitation, were calculated using data collected at the ECCC weather station in Fort McMurray (ID: 3062696; 56°39′04″ N, 111°12′48″ W), the station closest to all four study sites that recorded these data (https://weather.gc.ca/city/pages/ab-20_metric_e.html). We used site-specific hourly mean and minimum ambient temperatures (°C), and air concentrations of sulfur dioxide (SO_2) and total hydrocarbons (THC) (http://www.wbea.org/monitoring-stations-and-data/historical-monitoring-data) collected at the WBEA weather stations in closest proximity to OS1 (Muskeg River), OS2 (Buffalo Viewpoint, Lower Camp), and REF1 (Anzac), but not REF2 as there was no such station in sufficiently close proximity to provide meaningful information specific to REF2. Air concentrations of NO, NO_2, NO_X, and particulate matter ($PM_{2.5}$), recorded at the same WBEA weather stations, were also utilized for site-specific measures at OS1 and REF1.

2.3. Stable isotopes

Carbon (δ^{15}C) and nitrogen (δ^{13}N) stable isotopes were analyzed in dorsal feathers at the G.G. Hatch Isotope Laboratories (Ottawa, ON, Canada) as previously described (Fernie et al., 2017, Fernie and Marteinson et al., 2018). Briefly, feathers and standards were weighed, placed in an elemental analyzer interfaced to an isotope ratio mass spectrometer (IRMS) and flash-combusted at approximately 1800 °C (Dumas combustion). The resulting gas products were separated by a “purge-and-trap” adsorption column and sent to IRMS interface followed by IRMS. The data are reported in δ notation, and the unit of measurement, per mL (‰), defined as δX = [(Rsample − Rstandard)/Rstandard]*1000; X is the δ13C or δ15N values, and R the corresponding ratio of 13C/12C or 15N/14N. The δ15N values are reported as ‰ versus atmospheric nitrogen (AIR) and normalized to internal standards calibrated to international standards (i.e., IAEA-N1 (+0.4‰), IAEA-N2 (+20.3‰), USGS-40 (−4.52‰), USGS-41 (47.57‰)). The δ13C values are reported as ‰ vs. V-PDB and similarly, normalized to internal standards calibrated to international standards (i.e., IAEA-CH-6 (−10.4‰), NBS-22 (−29.91‰), USGS-40 (−26.24‰), USGS-41 (37.76‰)). Measurement precision was estimated to be 0.08‰ and 0.04% for 13 °C and 15°N, respectively.

2.4. Analysis of PAC concentrations in nestling feces and muscle

As previously reported (Fernie and Marteinson et al., 2018), PAC concentrations were assessed in fecal samples (2012, 2013) and muscle tissues (2013 only) collected from the same nestlings as in the present study. Briefly, after the samples were homogenized and spiked with a surrogate standard mixture of deuterated PAHs (d-PAHs), they were extracted by accelerated solvent (ASE 350, Dionex, Sunnyvale, CA, USA) with dichloromethane or DCM (100 °C, 1500 psi). After lipid content determination, the remaining extract was loaded on a 2-g Isolute® silica solid phase extraction (SPE) column. The silica sorbent was pre-cleaned with DCM to remove potential contamination and the packed SPE column was conditioned with 10 mL hexane (HEX). After loading the sample followed by 3 mL HEX, the SPE cartridge was eluted with 11 mL of a mixture of DCM and HEX (40:60, v/v) which contained analytes of interest and was concentrated and spiked with an internal standard d14-dibenzo(a,i)pyrene (Toronto Research Chemicals, Toronto, Canada). Separation and quantification of target PACs was conducted with an Agilent 7890 gas chromatograph (GC; Agilent Technologies, Palo Alto, CA) coupled with a single quadrupole mass analyzer (Agilent 5977A MS) in the electron impact (EI) and selected ion monitoring (SIM) mode. The 30 m HP-5MS column (0.25 mm i.d., 0.25 µm, J&W Scientific, Agilent Tech.) was used and the injector was operated in pulsed-splitless mode.

Our QA/QC measures included analysis of Standard Reference Materials (SRMs), spiking experiments, examination of surrogate standard recoveries, and process of procedural blanks as previously reported (Fernie and Marteinson et al., 2018). From the spiking experiments, recoveries (mean ± standard deviation) of individual PAC analytes ranged from 75 ± 5% to 94 ± 5%. Concentrations of PACs ranged from 75 ± 4% to 107 ± 4% of the certified concentrations in the NIST SRM 1974c (mussel tissue; *Mytilus edulis*) included in every three batches of authentic samples. A sum PAC concentration of 0.3–1.35 ng/g was determined in procedural blanks run along with each set of 5 samples; they were then subtracted from the concentrations measured in authentic samples. PAC concentrations in the nestling samples were corrected with surrogate standard recoveries (70 ± 4% to 91 ± 6%). The limit of detection for PACs ranged from 0.05 to 0.15 ng/g ww.

As previously described (Fernie and Marteinson et al., 2018), we calculated sum (Σ) concentrations of Σalkylated PAHs (Σalkyl-PAHs: C1,4-naphthalenes, C1-3-fluorenes, C1-4-phenanthrenes, C1-4-fluoranthenes/pyrenes, C1-4-benz(a)anthracenes/chrysenes), Σparent PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, 1,2-benzofluorene, 2,3-benzofluorene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k,i)fluoranthene, benzo(e)pyrene, benzo(g,h,i)perylene), and Σdibenzothiophenes (ΣDBTs: dibenzothiophene, C1-4-dibenzothiophenes). We refer to six major PACs, specifically naphthalene, C1- and C2-naphthalenes, C1- and C2-fluorenes, and C1-phenanthrenes, that were measured in at least 60% of the nestlings (Custer et al., 2017).

2.5. Statistical methods

The statistical analysis was conducted with IBM SPSS© 23, and when applicable, we used a statistical significance level of $p \leq 0.05$ and identified statistical trends with p-values > 0.05 and ≤ 0.08. Data were first tested for normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene's Test). Data that met these conditions either immediately or after log-transformation, were analyzed using parametric statistical tests; otherwise, non-parametric statistical methods were utilized. Data were not transformed for general linearized mixed models (GLMMs).

Reproductive differences among the study sites (clutch initiation and hatch dates, offspring numbers, overall reproductive success) were examined using Kruskal-Wallis statistical tests. Differences in nestling size (mass, wing chord and feather length, condition) and growth among the study sites were analyzed using GLMMs with linear models (as data were normally distributed), with data categorized by brood (to account for relatedness, dietary differences, sibling competition), and models including the fixed factors of site, year and hatching date, and the random factor of brood. Only models in which all factors were significant (i.e., confidence intervals did not cross zero) are reported. Ambient temperatures, wind speed and total precipitation were compared among the study sites using Analysis of Covariance (ANCOVA), with the covariate of Julian hatch date used to incorporate temporal effects. Differences in VOC measures between REF1 and OS1 were analyzed using Mann-Whitney U tests. As we previously reported (Fernie and Marteinson et al., 2018), significant differences among the study sites in feather stable isotope values were conducted by year using Kruskal-Wallis tests (δ^{15}N - 2013) or ANCOVAs with Julian lay date as a covariate (δ^{13}C) to address seasonal changes.

To identify factors that likely influenced the number of fledglings each pair produced, a series of GLMMs were conducted and ranked using Akaike's Information Criterion corrected for small sample sizes (AIC_C) using the most complete data set (REF1, OS1, OS2). The data had a Poisson distribution (determined by Kolmogorov-Smirnov test) and were modeled as such and categorized by site. All models included the random effect of site and its intercept to account for variability among sites, and up to three fixed factors that are known to influence avian reproduction or development in general. These fixed factors included weather variables (i.e., mean or minimum hourly ambient temperatures, mean hourly wind speed, total precipitation or number of rainy days or heavy rain days), VOC measures (SO_2, THC), Julian lay date, stable isotope values, and concentrations of ΣPACs, Σparent-PAHs, Σalkyl-PAHs, ΣDBTs and the 6 major PAHs, measured in the same individual nestlings. All other reproductive measures were not examined because the distributions of these data excluded using GLMMs.

To determine possible factors related to nestling body weight at d 9 or d 14, a series of GLMMs were conducted with data categorized by brood and ranked by AIC_C. All models included the random factor of brood and its intercept and up to three fixed factors known to influence nestling growth including site (all four sites), sex, Julian hatch date, brood size, regional total precipitation, number of rainy or heavy rain days during brood rearing, stable isotopes in feathers, and concentrations of major PAHs and sum PAC measures. Linear models were used as data were normally distributed.

For each of the GLMM analysis of fledgling numbers and nestling body weight, model selection was conducted with both years combined (year as a fixed factor) to address the birds' exposure to PACs (fecal concentrations), and then for 2013 only to further address the accumulation of PACs (muscle) and the particularly inclement weather that occurred that year. Only the better-performing models of those with redundant variables were retained (fecal vs. muscle PAC concentrations, sum PAC measures, weather). Only models ranking above the null model and for which all factors were significant are presented. The ΔAIC_C and Akaike's weights (w_i) were calculated and used to identify the best models.

3. Results

The exposure and accumulation of six major PAC congeners and related summed PAC concentrations by the nestling tree swallows varied significantly across the study sites (Fernie and Marteinson et al., 2018) (Fig. 2).

3.1. Reproductive measures, growth and diet

During the first year of the study, nest box occupancy by the tree swallows was low (≤10%) as expected, but improved in the second year to the following: OS1 (35%), OS2 (15%), REF1 (22%). In 2012 only, a trend was evident whereby clutch initiation began earlier for tree swallows at OS2 than at OS1 or REF1 ($X^2 = 5.55$, $p = 0.06$) (Fig. 3) (Table 1). Clutch sizes were similar among all pairs in 2012 ($p = 0.60$) (Table 1). Reflecting their earlier start to egg laying, the birds at OS2 completed their clutches ($X^2 = 6.77$, $p = 0.03$) and began incubation before the other birds ($X^2 = 5.55$, $p = 0.03$) (Table 1) (Fig. 3). Hatching dates, egg-laying patterns, and the length of the egg-laying and incubation periods were comparable across all four study sites (p-values ≥ 0.09) (Table 1).

Overall reproductive success significantly differed across the four study sites in 2013 ($X^2 = 8.09$, $p = 0.04$) (Fig. 4), with the swallows at OS2 having markedly lower success (65%) than those at OS1 (89%) or the reference sites (REF1: 79%; REF2: 88%). In 2013, there was limited evidence of site differences in hatchling ($X^2 = 6.80$, $p = 0.08$) and fledgling production by each pair ($X^2 = 6.89$, $p = 0.08$) (Fig. 5), but no differences in hatching or fledgling success (Table 1) despite the decline between the two stages for birds breeding at OS2 (Fig. 5). As previously reported (Cruz-Martinez et al., 2015), reproductive performance was similar among the pairs at OS1, OS2 and REF1 in 2012 (Table 1).

With both years combined, the body weight of the younger chicks at d 9 significantly differed among the sites ($F_{3,239} = 7.26$, $p < 0.001$) and was significantly influenced by their hatch date ($F_{1,239} = 11.10$, $p = 0.001$). The body weight of the older chicks (d 14) also significantly differed among the study sites ($F_{3,238} = 4.44$ $p = 0.005$; brood: $F_{1,238} = 6.16$ $p = 0.01$) with a significant effect of year ($F_{1,238} = 12.32$, $p = 0.001$). At both ages, nestlings at OS2 were lighter than the other nestlings. Weight gain between d 9 and d 14 were similar for the chicks among the study sites (Fig. 6). Feather length and growth from d 9 to d 14 did not vary among the study sites. The condition of younger chicks (d 9) varied among the study

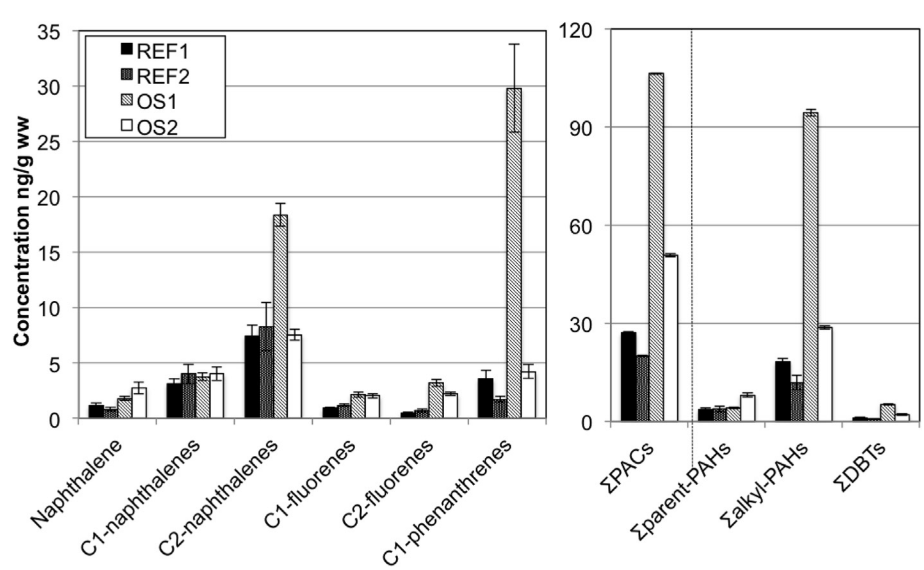

Fig. 2. Muscle concentrations of "major" PACs and sum measures of total PACs, parent and alkylated (alkyl) PAHs and dibenzothiophenes (DBTs) in 14 d old nestling tree swallows in the AOSR Fernie and Marteinson et al. (2018).

Table 1
Overall comparisons of reproductive parameters of tree swallows nesting at reference sites (REF1, REF2) and mining-related sites (OS1, OS2) in the Athabasca Oil Sands Region (Kruskal-Wallis Tests). (*) = marginally significant *p*-value; NS = not significant. Measures of reproductive success from 2012 were previously published and group comparisons with Generalized Estimating Equations yielded the same results (Cruz-Martinez et al., 2015). Stable isotope values for 2013 were published in Fernie and Marteinson et al. (2018).

		2012							2013								
		REF1		OS1		OS2		*p*-value	REF1		REF2		OS1		OS2		*p*-value
		Mean	SE	Mean	SE	Mean	SE		Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Sample size	# pairs breeding (N = 47)	6		3		6			9		4		13		6		
Julian start date	1st egg laid (d)	150	0.9	152	1.5	146	1.2	0.06(*)	154	3.4	164	7.5	151	2.5	153	3.6	NS
	Last egg laid (d)	156	0.6	156	1	152	1.2	0.03	159	3.2	167	7.1	156	2.2	158	3.2	NS
	Incubation (d)	157	0.6	158	1	153	1.2	0.03	162	3	169	6.5	158	2.1	160	1.7	NS
	1st Hatch (d)	170	0.8	170	2	165	1.5	NS	174	3	181	6.5	169	1.9	171	3.1	NS
Duration	Egg-laying (d)	6	0.5	5	0.6	6	0.3	NS	5	0.2	4	0.5	5	0.5	6	0.5	NS
	Incubation (d)	13	0.3	13	1	12	0.4	NS	12	0	12	0	12	0.4	11	0.5	NS
Laying pattern	# Eggs laid/d	1	0.1	1	0.02	1	0.01	NS	1	0.01	1	0.1	1	0.1	1	0.05	NS
Reproduction	Clutch size	6	0.4	6	0.6	7	0.2	NS	6	0.2	5	0.5	6	0.3	6	0.3	NS
	# Hatchlings/brood	6	0.6	6	0.9	6	0.3	NS	5	0.3	4	0.4	5	0.6	6	0.3	0.08(*)
	Hatching success (%)	89	6	93	7	97	3	NS	87	5	88	13	89	9	90	4	NS
	# Fledglings/brood	6	0.6	6	0.9	6	0.2	NS	5	0.4	4	0.4	5	0.6	4	0.8	0.08(*)
	Fledging success (%)	100	0	100	0	100	0	NS	92	5	100	0	100	0	75	15	NS
	Overall success (%)	89	10	93	10	97	3	NS	79	10	88	10	89	10	65	10	0.04
Sample size	# nestlings (N = 116)	16		8		18			24		9		23		18		
Stable isotopes	$\delta^{13}C$ – feathers	−28.50	0.40	−25.10	0.50	−27.50	0.30	≤0.001	−30.20	0.02	−25.5	0.40	−29.4	0.50	−27.50	0.40	<0.001
	$\delta^{15}N$ – feathers	7.90	0.20	8.40	0.10	7.30	0.10	≤0.001	7.70	0.20	7.80	0.03	6.90	0.10	7.20	0.10	0.002

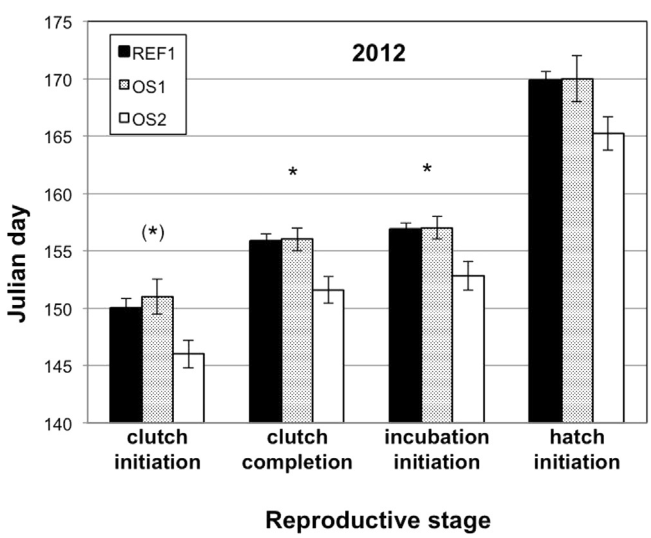

Fig. 3. Timing of key reproductive measures of tree swallows nesting at mining-impacted sites (OS1: N = 3; OS2: N = 6) and a reference site (REF1: N = 6) in the Athabasca Oil Sands Region of northern Alberta, 2012. Raw means for respective Julian calendar dates are presented, and statistical significance was determined using Kruskal-Wallis tests. **P*-values < 0.05; (*) *p*-value = 0.06 (first egg).

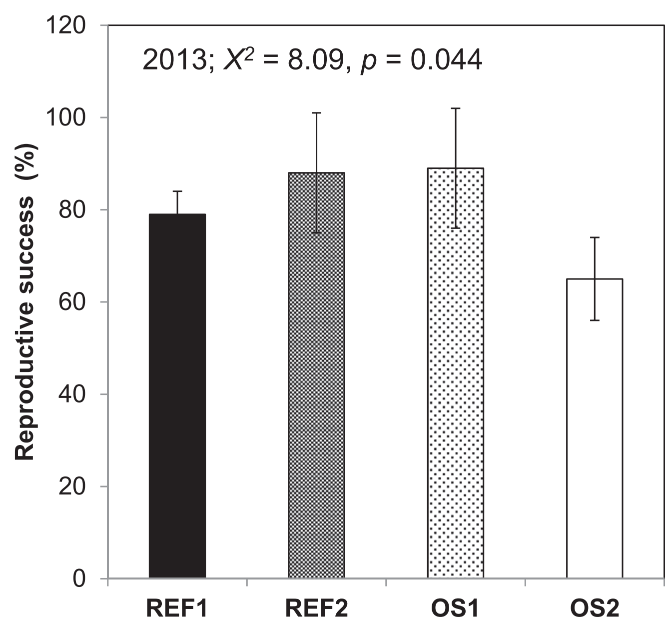

Fig. 4. Overall reproductive success (percent of eggs that fledged per pair) of tree swallows differed significantly among mining-impacted sites (OS1: N = 13; OS2: N = 6) and reference sites (REF1: N = 9; REF2: N = 4) in the Athabasca Oil Sands Region, 2013.

sites ($F_{3,240} = 9.09$, $p \leq 0.001$) and between years ($F_{1,240} = 11.24$, $p = 0.001$), with younger chicks at OS2 being markedly lighter relative to their size. There were no obvious physical signs of disease or deformities in the nestlings at d 14.

A detailed description of the statistical analysis of the feather isotope values as proxies of diet is provided in the Supplementary Information (SI) section. In 2012, feather $\delta^{15}N$ and $\delta^{13}C$ values of the nestlings significantly differed among the study sites (all *p*-values ≤ 0.001) (Table 1), with a significant effect of the progressing season (i.e., clutch initiation) on $\delta^{15}N$ values ($F_{1,38} = 48.0$ $p < 0.001$) (Table 1). Feather $\delta^{15}N$ values were highest in the birds' feathers from REF1, followed by OS2 and then OS1 (LSD: *p*-values ≤ 0.03). Feather $\delta^{13}C$ values were highest in birds at OS1 then REF1 followed by OS2 (LSD: *p*-values ≤ 0.01). In 2013, feather $\delta^{15}N$ values were significantly higher at OS1 and OS2 than the two reference sites, while feather $\delta^{13}C$ values were similar at REF1 and OS1, but lower than at REF2 or OS2 (Fernie and Marteinson et al., 2018).

3.2. Sites differences in weather and air quality measures

A detailed description of the significant differences among the study sites in weather and VOC variables is also provided in the SI. Briefly, ambient temperatures were significantly warmer at OS1 than REF1 or OS2 prior to egg laying (both years) and the 2013 nestling period (*p*-values ≤ 0.006) (Table S1). It was significantly

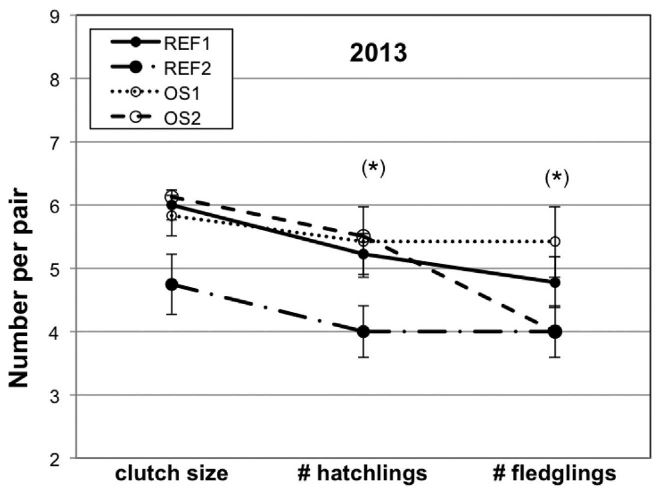

Fig. 5. Reproductive measures demonstrating the number of potential offspring per pair of tree swallows nesting at mining-related sites (OS1: N = 13; OS2: N = 6) and reference sites (REF1: N = 9; REF2: N = 4) in the Athabasca Oil Sands Region in 2013. Note the decline from hatchling production to fledgling production for pairs breeding at OS2. (*) *p*-values = 0.08.

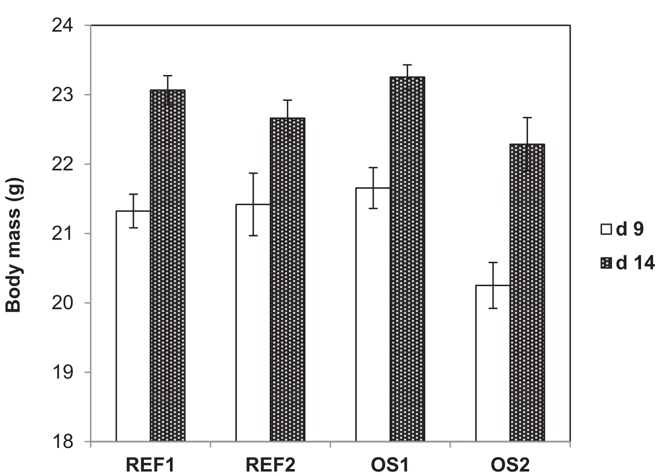

Fig. 6. The body weight of nestling tree swallows was significantly different at d9 ($F_{3,239} = 7.26$, N = 99) and d14 ($F_{3,238} = 4.44$ $p = 0.005$, N = 106) among the reference (REF1, REF2) and mining-impacted (OS1, OS2) study sites in the Athabasca Oil Sands Region (2012, 2013 combined).

windier at OS2 prior to egg laying (*p*-values < 0.001), but windiest at OS1 during brood rearing (*p*-values < 0.001) (Table S1). In 2013, total regional precipitation significantly differed among the study sites during brood rearing (*p*-values < 0.001): less rain fell at OS2 than the other sites where rainfall was similar. Ambient temperatures, wind and regional precipitation significantly varied over the course of the brood rearing period (Julian hatch date; *p*-values ≤ 0.001). With the exception of PM2.5 (2012) and NO (2013), all of the other VOC measures were significantly higher at OS1 than REF1 before egg laying began (*Z*-values ≤ −3.85, *p*-values ≤ 0.02), and during brood rearing (except SO_2 in 2013), with greater differences evident between OS1 and REF1 in 2013 (*Z*-values ≤ −3.85, *p*-values ≤ 0.001) (Table S1).

3.3. Model selection for fledgling production and nestling development

Some of the variation in fledgling production by each pair (both years combined) was explained by the hatch date of the chicks ($p < 0.001$) but not by any other measured fixed factors (Table 2). In 2013, several models explained a proportion of the variation in fledgling production (Table 2). The two best models included first, only the lay date ($w_i = 0.35$; negative relationship), and then, total precipitation (positive relationship) and muscle concentrations of C1-napthalenes combined (negative relationship; $w_i = 0.19$; Table 2). Fecal ΣDBTs ($p = 0.04$; positive relationship) and $\delta^{15}N$ values ($p = 0.03$; negative relationship) were also significantly related to fledgling production (Table 2). Concentrations of other PACs, VOCs, and wind speed, were unrelated to the number of fledglings produced in each brood.

When 2012, 2013 were considered together, the variation in body weight of the younger chicks (d 9) was best explained ($w_i = 0.72$) by study site and fecal C1-phenanthrenes (positive relationships) combined, followed by a fairly weighted model ($w_i = 0.27$) including fecal C2-fluorenes (negative relationship) and fecal C1-phenanthrenes (positive relationship) (Table 2). The $\delta^{13}C$ values of the nestlings were also negatively related to their body mass on d 9 ($p = 0.03$; Table 2). For the older chicks (d 14), body weight was best explained ($w_i = 0.99$) by the year (nestling being heavier in 2012) and sex of the nestlings (males being heavier; Table 2); total regional precipitation during brood rearing ($p = 0.007$, negative relationship), was also significantly related to body mass of the older chicks (d 14) (Table 2).

In 2013, body weight of younger nestlings (d 9) was best explained by the model including study site, the number of rainy days during brood rearing, and the accumulation (muscle concentrations) of C1-phenathrenes by the nestlings ($w_i = 0.54$) (Table 2). The subsequent and fairly weighted model ($w_i = 0.18$) included muscle Σalkyl-PAHs (Table 2). Muscle concentrations of ΣDBTs were also positively related to the body weight of nestlings on d 9 (*p*-values ≤ 0.04) (Table 2). In all of the models, muscle concentrations of the measured PACs outperformed fecal concentrations in explaining the body weight of chicks. The body weight of older nestlings (d 14) was significantly related to the number of rainy days during brood rearing, hatch date, and the study site (Table 2).

4. Discussion

This study demonstrates that differences in the timing of clutch initiation, reproductive success, nestling growth (weight) and body condition, can occur and may vary inter-annually for birds breeding in close proximity to mining-related activity in the AOSR compared to those breeding at reference sites in the region. At one of the mining-related sites, OS2, there was a trend towards earlier clutch initiation in 2012 that contributed to significantly earlier clutch completion and commencement of incubation. Although advanced egg-laying is often associated with better reproductive success in temperate breeding birds, including tree swallows (Winkler et al., 2011 and references therein), reproductive success was similar across all pairs of swallows that year. In the second year of study (2013), the timing of breeding was similar for the birds at all sites, yet the birds at OS2 had significantly lower overall reproductive success (65%) than those at the other three sites (79–89%) reflecting the decline in nestling production between hatching and fledging at OS2 (Fig. 5). Moreover, tree swallow nestlings at OS2 were lighter and in poorer condition close to fledging. The stable isotope results suggest differences occurred among the study sites and between years in nestling diet relating to dietary sources (e.g., terrestrial, aquatic) and the trophic position of insects ($\delta^{15}N$) consumed by chicks. The timing of egg laying, the nestlings' exposure and accumulation of specific alkyl-PAHs and DBTs, their diet (dietary sources and trophic position), and the extent of rainfall during brood rearing, were related, likely through complex

Table 2
Factors influencing fledgling production and body weight of tree swallows in the Athabasca Oil Sands Region, Canada.

Response variable	Model	ΔAICc	w_i	B	p	95% confidence intervals
# Fledglings/pair 2012 + 2013	clutch initiation date	0	1	−0.1	<0.001	−0.2 − −0.1
	null	5.2				
# Fledglings/pair 2013	clutch initiation date	0	0.35	−0.1	<0.001	−0.2 − −0.1
	total precipitation + m C1-naphthalene	1.2	0.19	0.4, −0.4	0.001, 0.007	0.2–0.5; −0.7 − −0.1
	m C1-naphthalene + f ΣDBTs	2.2	0.12	−0.4, 0.6	0.007, 0.01	−07 − −0.1; 0.1–1.0
	total precipitation	2.2	0.11	0.3	0.004	0.1–0.5
	δ^{15}N + m C1-naphthalene	3.3	0.07	−0.4, −0.4	0.024, 0.028	−0.8 − −0.1; −0.7 − −0.04
	δ^{15}N	3.6	0.06	−0.4	0.032	−0.7 − −0.03
	f ΣDBTs	3.8	0.05	0.5	0.04	0.02–0.9
	m C1-naphthalene	4.0	0.05	−0.3	0.044	−0.65 − −0.01
	null	4.24				
Nestling weight (d 9) 2012 + 2013 n = 99	site + f C1- phenanthrene	0	0.72	site 1–4: 2.3, 2.4, 0.5, 0; 0.2	site 1–3: <0.001, 0.007, 0.51; <0.001	site 1–3: 1.2–3.5, 0.7–4.2, −0.9–1.8; 0.1 −0.4
	f C2-fluorene + f C1-phenanthrene	2	0.27	−0.7, 0.2	<0.001, <0.001	−1.1 − −0.4; 0.1–0.4
	site	9	0.01	site 1–4: 2.0, 2.0, 1.5, 0	site 1–3: 0.002, 0.035, 0.020	site 1–3: 0.8–3.2, 0.1–3.8, 0.2–2.8
	f C2-fluorene	11.8	0	−0.455	0.016	−0.8 − −0.09
	δ^{13}C	13.6	0	−0.286	0.028	−0.5 − −0.03
	f C1- phenanthrene	14.6	0	0.146	0.023	0.02–0.3
	null	16.2				
Nestling weight (d 9) 2013 n = 64	m C1-phenanthrene + site + rainy days	0	0.54	0.37; site 1–4: 1.3, 2.4, 0.5, 0; 0.35	0.01; site 1–3: 0.04, 0.01, 0.5; 0.04	0.02–0.1; site 1–3: 0.1–2.7, 0.8–4.1, −1.2 − 2.3; 0.02–0.7
	m Σalkyl-PAH + site + rainy days	2.2	0.18	0.03, site 1–4: 1.6, 2.7, 0.4, 0; 0.4	0.01; site 1–3: 0.01, 0.002, 0.63; 0.03	0.001–0.05; site 1–3: 0.3–2.9, 0.02 −0.7, −1.4–2.3; 0.04–0.7
	m C1-phenanthrene + site	2.7	0.14	0.08, site 1–4: 1.4, 2.1, 1.6, 0	0.003; site 1–3: 0.04, 0.01, 0.6	0.03–0.1; site 1–3: 0.03–0.1, 0.1–2.7, 0.4 −3.8, −1.3–2.3
	m ΣDBT + site	4.3	0.06	0.3; site 1–4: 1.6, 2.3, 0.5, 0	0.04; site: 1–3: 0.02, 0.01, 0.6	0.01–0.5; site 1–3: 0.2–3.0, 0.5–4.0, 0.1 −3.2
	m Σalkyl-PAH + site	5.2	0.04	0.03; site 1–4: 1.7, 2.4, 0.5	0.01; site 1–3: 0.02, 0.01, 0.6	0.01–0.1; site 1–3: 0.01–0.5, 0.3–3.0, 0.7 −4.1
	# rainy days + site	6	0.03	0.5; site 1–4: 1.7, 2.4, 1.9, 0	0.002; site 1–3: 0.06, 0.01, 0.01	0.2–0.8; site 1–3: 0.01–0.1, −0.1–2.7, 0.7 −4.1
	m C1- phenanthrene	13.8	0	0.06	0.001	0.03–0.1
	site	14.1	0	site 1–4: 1.4, 1.9, 2.1, 0	site 1–3: 0.07, 0.04, 0.01	site 1–3: −0.1–2.9, 0.1–3.7, 0.6–3.6
	m ΣDBT	15.8	0	0.25	0.02	0.1–0.5
	m Σalkyl-PAH	18.3	0	0.2	0.003	0.01–0.03
	rainy days	18.4	0	0.5	0.004	0.2–0.8
	m C2-napthalene	19.3	0	0.1	0.04	0.003–0.2
	null	25.2				
Nestling weight (d 14) 2012 + 2013 n = 106	year + sex	0	0.99	2013: −0.9, F: 0.9	2013: 0.026, F: 0.018	2013: −1.6 − −0.1; F: 0.2–1.5
	year + hatch date	10.8	0	2013: −1.1; 0.06	2013: 0.005; 0.011	2013: −1.9 − −0.3; 0.01–0.1
	year	11.6	0	2013: −0.84	2013: 0.029	2013: −1.6 − −0.1
	total precipitation	13.1	0	−0.14	0.007	−0.3 − −0.04
	null	16.4				
Nestling weight (d 14) 2013 n = 72	heavy rain d + site	0	0.74	−0.5; site 1–3: 0.7, 0.7, 1.3, 0	0.003; site 1–3: 0.01, 0.02, 0.001	−0.9 - − 0.2; site 1–3: 0.2–2.0, 0.3–2.5, 0.7–2.5
	hatch date + site	2.4	0.22	0.1; site 1–3: 0.7, 0.7, 1.3	0.001; site 1–3: 0.1, 0.3,0.003	0.03–0.1
	# nestlings	9	0.01	−0.5	0.003	−0.7
	site	7	0.02	site 1–3: 0.8, 1.3, 1.3, 0	site 1–3: 0.06, 0.03, 0.009	1–3: −0.1–1.8, 0.1–2.5, 0.3–2.2
	hatch date	11.9	0	0.1	0.001	0.03–0.1
	hatch date + m C1-phenanthrene	12.1	0	0.1; 0.02	0.001, 0.04	0.03–0.1, 0.001–0.04
	heavy rain d	13.3	0	−0.4	0.03	−0.7 - −0.03
	null	16.2				

Factors were identified using Generalized Linear Mixed Models ranked by Akaike's Information Criterion corrected for small sample sizes (AIC_C). ΔAICc: the difference between a model and the best model; w_i: Akaike weight; B: coefficient; p, 95% confidence intervals for each fixed factor presented in order. Clutch initiation date = Julian date that first egg of clutch was laid; total precipitation, rainy days (# of rainy d ≥ 1–9.9 mm), heavy rain days (≥10 mm) determined during brood rearing; m = muscle concentrations, f = fecal concentrations; "Site": site 1 = REF1; site 2 = OS1; site 3 = OS2; site 4 = REF2.

interactions and relationships, to reproductive and developmental changes in the tree swallows.

4.1. Clutch initiation

The timing of clutch initiation, and the age and prior breeding experience of adult birds are critical factors in determining the reproductive success of seasonally breeding birds. Generally, older birds with prior breeding experience lay their eggs earlier and have better reproductive success. The timing of clutch initiation has evolved to coincide with the most favorable ecological conditions for nestling survival (reviewed in Verhulst and Nilsson, 2008). In most species, including tree swallows, the earlier birds breed, the greater their success (Winkler et al., 2011 and references therein), but any changes in the timing of egg laying will likely negatively affect this balance. We identified that the start of clutch initiation was one of several factors influencing fledgling production, and that clutch initiation began earlier for the tree swallows at the one mining-related site (OS2) compared to the other sites in 2012.

Multiple environmental and ecological factors contribute to when birds are capable of reproduction and thus determining the onset of clutch initiation. Prior to egg-laying, the swallows at the mining-impacted sites experienced warmer (OS1) and windier (OS2) conditions than the reference birds. Ambient temperatures and wind conditions can alter food availability (reviewed in Winkler et al., 2011) and food availability is likely the main determinant of clutch initiation by tree swallows (Dunn et al., 2011; reviewed in Winkler et al., 2011). Altered food availability from the warmer, windier conditions on the OS sites in our study may be reflected by the significant site differences in stable isotope values that are proxies of the tree swallows' diet. Feather $\delta^{15}N$ values suggested that the trophic position and hence composition of the birds' diet differed among the sites, potentially reflecting to differences in prey availability during the breeding season.

Clutch initiation is also determined by intrinsic factors involving reproductive physiology, hormones, and courtship behaviors (reviewed in Winkler et al., 2011; Dawson et al., 2001; McNabb, 2007). Many organic contaminants (e.g., polychlorinated biphenyls, brominated flame retardants) alter the timing of breeding in birds, including advances and delays in clutch initiation, and affect gonadal physiology, reproductive hormones, and courtship behaviors (Fernie et al., 2008; Marteinson et al., 2010, 2012, 2015). While there is no information available on potential reproductive effects of DBTs or alkyl-PAHs, the parent PAH, naphthalene, impaired sexual maturation and seasonal ovarian growth (Thomas and Budiantara, 1995), reduced concentrations of sex hormones (Tintos et al., 2006, 2007), and thus has potential to influence the onset of clutch initiation and reproduction. Here, reproductive changes occurred in the same tree swallows whose nestlings were exposed to and accumulated higher concentrations of naphthalene, and even higher concentrations of C1- and C2-naphthalenes and C1-phenanthrenes on the OS sites (Fernie and Marteinson et al., 2018). Further research is required to determine if exposure to PACs, particularly alkyl-PACs, may affect reproductive systems and influence the timing of clutch initiation and reproduction of adult birds, as demonstrated by the present tree swallows at one mining-impacted site.

4.2. Reproductive success

In other studies with tree swallows in the AOSR, reproductive success has varied among years, with adverse reproductive changes observed for swallows in some but not all years (e.g. Smits et al., 2000; Gentes et al., 2006 and this study). In the present study, fledgling production and reproductive success of the tree swallows in the AOSR varied among years: tree swallows produced approximately two more fledglings per pair in 2012 than 2013 (Table 1) and experienced much poorer reproductive success (65%) at OS2 than at other study sites ($\geq$79%) in 2013.

In our study, we identified that the fledgling production by the swallows was influenced by multiple factors or stressors, namely the timing of clutch initiation, their exposure and accumulation of specific PACs, their diet, and the rainfall they experienced during brood rearing. As anticipated, the influence of some of these multiple stressors varied by year. The timing of clutch initiation was the most influential factor determining fledgling production of the tree swallows in 2012 and 2013 (combined): simply, clutches that were initiated earlier were more likely to survive to fledging, resulting in better fledgling production, consistent in general for this and other temperate breeding species (Winkler and Allen, 1996 and references therein). In 2013, 100-year record rainfalls, flooding and cold temperatures occurred during brood rearing, and it was these heavy rains, the timing of egg laying, the varying composition of the nestlings' diet ($\delta^{15}N$: trophic position of prey), and their exposure to ΣDBTs and accumulation of C1-naphthalenes, that most influenced fledgling production. Our results suggest that as the nestlings accumulated more C1-naphthalenes and their diet consisted increasingly of higher trophic insects ($\delta^{15}N$), there was a corresponding decline in fledgling production, suggesting a negative impact on nestling health and survival. Our findings are consistent with previous findings (Gentes et al., 2006) in which tree swallows nesting at reclaimed wetlands in the AOSR had higher or complete mortality (60–100%) compared to the reference birds in a season with highly adverse weather conditions. Excessive rain, cold ambient temperatures and rapid, large decreases in ambient temperatures (e.g., decrease of 16 °C) (Smits et al., 2000), are known to adversely affect nestling growth and survival (McCarty and Winkler, 1999).

4.3. Nestling growth

Avian growth is also affected by organic pollutants, diet, and adverse weather. Previous studies have shown that birds exposed to PAHs (reviewed in Albers, 2006), as well as birds raised in the AOSR (Smits et al., 2000; Gurney et al., 2005), may experience inhibited growth and development. A large number of laboratory PAH exposure studies in which chicken embryos were exposed by egg injection or coating of the egg, showed that PAH mixtures caused mortality, liver necrosis, and reduced growth (reviewed in Albers, 2006). When chicks were exposed to crude oil, growth rates and body size were reduced, multiple organs increased in mass (e.g., liver, adrenals), and circulating corticosterone and thyroxine concentrations increased (reviewed in Albers, 2006). In the AOSR, the body weight of tree swallow nestlings was reduced at one reclaimed wetland (Smits et al., 2000), and mallard ducklings were lighter and smaller (shorter tarsus and bills) when raised on wastewater collected in the region (Gurney et al., 2005).

In the present study, the tree swallow nestlings showed no overt physical signs of disease or deformities. However, the chicks at the one mining-related site (OS2) were in poorer condition and were lighter when younger (d 9) and as they approached fledging (d 14). Similarly, deer mice were also in poorer condition on a reclaimed mining site in the AOSR (Rodriguez-Estival and Smits, 2016). Based on our modeling, the nestlings' exposure to C1-phenanthrenes and C2-fluorenes, and the source of their diet ($\delta^{13}C$; terrestrial vs. wetland), contributed to the variation in the body weight of the younger chicks (d 9) but not of the older chicks (d 14). The positive relationship between the exposure of the younger chicks to C1-phenanthrenes and their body weight may initially be counter-intuitive since it suggests that they were heavier when exposed to higher concentrations of C1-phenanthrenes. However, exposure of animals to organic pollutants such as flame retardants has resulted in weight gain and the classification of such pollutants as obesogens and endocrine disruptors [e.g., Patisaul et al., 2013]. That the associations with chick body weight and C1-phenanthrenes (positive) and C2-flurorenes (negative) are opposite in direction, suggests complex relationships involving potential differential influences of individual PAC congeners and/or synergistic or additive effects that require further study. In the present study, the weight of the older nestlings (d 14) depended on their sex, when they hatched, and the extent of rainfall that occurred when parents were feeding their nestlings, and appears to be unrelated to their exposure and uptake of PACs. Collectively, these results suggest that there are different factors that influence chick body mass at different ages: diet and PACs may have more influence on younger chicks, while sex, hatch date and the extent of rainfall, but potentially not exposure to PACs, may influence the growth of older chicks.

Since rainfall influences the availability and types of aerial insects available for consumption by the nestling swallows, the positive relationship between body mass of the chicks and rainfall may be related to dietary sources ($\delta^{13}C$), availability, and timing of breeding. It appears that there was a possible difference in growth responses between male and female nestlings that warrants further investigation, and that the occurrence of developmental changes depended on the age of the nestlings (hatch date) when they were subjected to conditions resulting from the heavy rains. In addition, when highly inclement weather occurred during brood rearing (e.g., 2013), the mass of younger nestlings was likely influenced by the extent of rainfall and their exposure and accumulation of C1-phenanthrenes, Σalkyl-PAHs and ΣDBTs. Younger nestlings require parental incubation until they reach an age when they are capable of proper thermoregulation. We hypothesize that for nestlings in the AOSR, their increased exposure and accumulation of some PACs (Fernie and Marteinson et al., 2018), and dietary differences, may have sub-lethal effects on their growth and health that are exacerbated by adverse weather with its corresponding challenges of reduced availability of aerial insects for dietary consumption, and the inability of young nestlings to thermoregulate. Thermoregulation is partially governed by thyroid function in vertebrates, and we are currently investigating if thyroid function was altered in the present tree swallow nestlings.

5. Conclusion

The present study demonstrates that multiple and complex ecological, environmental and/or chemical (e.g., PACs) stressors contributed to perturbations in the reproduction and development of tree swallows when nesting in close proximity to mining-related activities in the AOSR. Adverse reproductive and developmental changes occur inconsistently, with the results of collective studies suggesting that they are more likely to happen during periods of highly inclement weather. The present study broadens our understanding that these changes occur during highly inclement weather in combination with changes in the birds' diet, and their exposure and accumulation of PACs through their diet, air (inhalation, deposition-preening of feathers), and mining-impacted freshwater sources at the breeding colonies (e.g., wetlands). Despite the advantage of slightly advanced clutch initiation, tree swallows at one mining-impacted site had comparatively lower overall reproductive success and chicks that were lighter and in poorer condition as they approached fledging. Suboptimal nestling growth and condition may contribute to reduced reproductive success of birds. Our results show that the birds' exposure and/or accumulation of specific parent- and alkyl-PAHs and DBTs may be important factors contributing to their reproductive and/or developmental changes, specifically naphthalene, C1-naphthalenes and ΣDBTs (fledgling numbers), and C1-phenanthrenes, C2-fluorenes, Σalkyl-PAHs and ΣDBTs (body weight at d 9); concentrations of these PACs were some of the highest (i.e., Σalkyl-PAHs, C1-phenanthrenes, C2-naphthalenes) and lowest (i.e., ΣDBTs, naphthalenes, C2-fluorenes) of those measured, to which these nestlings were exposed and/or accumulated (Fernie and Marteinson et al., 2018). Additionally, the diet of the nestlings and the extent of rainfall that occurred during brood rearing, were also important in relation to the birds' reproduction and development. Further investigations of other intrinsic mechanisms that potentially contributed to these reproductive and developmental changes in the tree swallows are currently underway, including the potential impacts on the thyroid function, stress and immunity of the same nestlings.

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2018.03.074.

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