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Migrating Tundra Peregrine Falcons accumulate polycyclic aromatic hydrocarbons along Gulf of Mexico following Deepwater Horizon oil spill

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Abstract Monitoring internal crude oil exposure can assist the understanding of associated risks and impacts, as well as the effectiveness of restoration efforts. Under the auspices of a long-term monitoring program of Tundra Peregrine Falcons (*Falco peregrinus tundrius*) at Assateague (Maryland) and South Padre Islands (Texas), we measured the 16 parent (unsubstituted) polycyclic aromatic hydrocarbons (PAHs), priority pollutants identified by the United States Environmental Protection Agency and components of crude oil, in peripheral blood cells of migrating Peregrine Falcons from 2009 to 2011. The study was designed to assess the spatial and temporal trends of crude oil exposure associated with the 2010 Deepwater Horizon (DWH) oil spill which started 20 April 2010 and was capped on 15 July of that year. Basal PAH blood distributions were determined from pre-DWH oil spill (2009) and unaffected reference area sampling. This sentinel species, a predator of shorebirds and seabirds during migration, was potentially exposed to residual oil from the spill in the northern Gulf of Mexico. Results demonstrate an increased incidence (frequency of PAH detection and blood concentrations) of PAH contamination in 2010 fall migrants sampled along the Texas Gulf Coast, declining to near basal levels in 2011. Kaplan–Meier peak mean $\sum$PAH blood concentration estimates varied with age (Juveniles-16.28 $\pm$ 1.25, Adults-5.41 $\pm$ 1.10 ng/g, wet weight) and PAHs detected, likely attributed to the discussed Tundra Peregrine natural history traits. Increased incidence of fluorene, pyrene and anthracene, with the presence of alkylated PAHs in peregrine blood suggests an additional crude oil source after DWH oil spill. The analyses of PAHs in Peregrine Falcon blood provide a convenient repeatable method, in conjunction with ongoing banding efforts, to monitoring crude oil contamination in this avian predator.

Keywords PAH · Tundra Peregrine Falcon · Sentinel species · Deepwater Horizon oil spill · Migration · Gulf of Mexico

Introduction

On a global scale, Peregrine Falcons (*Falco peregrinus*) are an apex predator and a documented sentinel species, sensitive to environmental contaminants and diseases in various avian communities that comprise their prey base (Hickey 1969; Ratcliffe 1980; Cade et al. 1988; Cade and Burnham 2003; Henny et al. 2009). Arctic populations of the Tundra Peregrine Falcon (*F. p. tundrius*) in North America were in steep decline by the early 1970s based on breeding ground surveys and observations made during migration to their southern wintering grounds. Breeding peregrines are difficult and expensive to access due to their remote nesting habitat, but peregrines of all ages and breeding status can be captured during autumn and spring migration. In the 1970s studies were begun at Assateague

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Island (Maryland and Virginia) and South Padre Island (Texas) to monitor the migrating Peregrine population and its level of contaminants. Migration biomonitoring provides a broad-scale, population level perspective of these remote nesting northern falcons that tracked the corresponding decline of DDT metabolite residues and located areas of continued exposure following associated bans on DDT use (Henny et al. 1982, 1988, 1996, 2009). Survey biomonitoring efforts have also illustrated the limited seroprevalence of avian Influenza A viruses in Nearctic Peregrines (Redig and Goyal 2012). Additionally, tissues from multiple years have been archived to address future contaminants, avian diseases of conservation and human health concern (Seegar et al. 2003) and genetic population assessments (Johnson et al. 2010).

Polycyclic aromatic hydrocarbons (PAHs) are globally distributed environmental contaminants derived from pyrogenic and petrogenic processes, originating from geophysical (e.g., crude oil seeps) and anthropogenic (e.g., oil spills) sources. Crude oil contains a mixture of PAHs, which are toxic to birds (Leighton 1993; Briggs et al. 1997). The kinetics of PAHs in the environment and biota are complex due to chemical weathering and PAH metabolism in vertebrates (Trust et al. 2000; Beyer and Meador 2011). Contaminated prey items are a potential PAH source for predators and the persistent incorporation of PAHs through trophic processes has been documented in avian species from exposure to residual crude oil after oil spills (Zuberogoitia et al. 2006; Alonso-Alvarez et al. 2007; Esler et al. 2010, Paruk et al. 2014).

Birds have well-developed mixed function oxygenase systems that can rapidly metabolize parent PAHs into hydrophilic products that are easily excreted. Consequently, only minor concentrations of parent compounds are usually detectable in avian tissues (Ariese et al. 1993; DiGuilio et al. 1995). Alternative monitoring techniques such as PAH metabolite bile burden and the induction of cytochrome P450 1A have been developed (Trust et al. 2000; Troisi et al. 2006). However, these techniques and liver tissue assays require freshly killed animals or intensive field surgeries (Esler et al. 2010; Rothscales et al. 2011; Velando et al. 2010). Recent non-destructive blood monitoring and dose–effect studies have addressed the potential for, and the sub-lethal effects of, persistent chronic exposure to low levels of petroleum hydrocarbons in seabirds (Troisi and Borjesson 2005; Alonso-Alvarez et al. 2007; Perez et al. 2008). The latter study spatially and temporally tracked a PAH pulse following a crude oil spill in Europe by red blood cell analyses and identified individual PAH trends in blood by an associated dose–effect study of Yellow-Legged Gulls (*Larus michahellis*). Since blood cells are continuously being produced and have a lifespan of several weeks, the presence of PAHs in red blood cells likely indicates recent incorporation during erythropoiesis (Clark 1988).

The 2010 Deepwater Horizon MC-252 (DWH) oil spill released an unprecedented 4.9 million barrels of crude oil into the northern Gulf of Mexico, impacting more than 1,046 km of intertidal beaches, mudflats and coastal wetlands (Graham et al. 2011). A recent study suggests movement of DWH carbon and PAHs from the planktonic food web to upper level trophic consumers (Mitra et al. 2011). Located in one of the world's largest migratory flyways, DWH oil spill generates concern of crude oil exposure and associated effects on migratory birds, while providing an opportunity to assess biomonitoring techniques and the avian trophodynamics of PAHs in associated coastal environments.

Tundra Peregrines concentrate on migration at Assateague and Padre Islands during movement from their nesting grounds across the Arctic (Alaska to Greenland) to wintering areas in the Mid-Atlantic states/Gulf Coast, south throughout Latin America (Fig. 1). Results from over four decades of banding data and two decades of telemetry tracking demonstrate that greater than 80 % utilize Gulf of Mexico coastal habitats as stopover and/or staging areas during migration (Yates et al. 1988; Enderson et al. 1995; Fuller et al. 1998; McGrady et al. 2002; Earthspan unpublished data). Migratory Peregrine prey includes shorebirds, seabirds, passerines and waterfowl (Hunt et al. 1975; Ward and Laybourne 1985; Russell 2005). Sub-surface

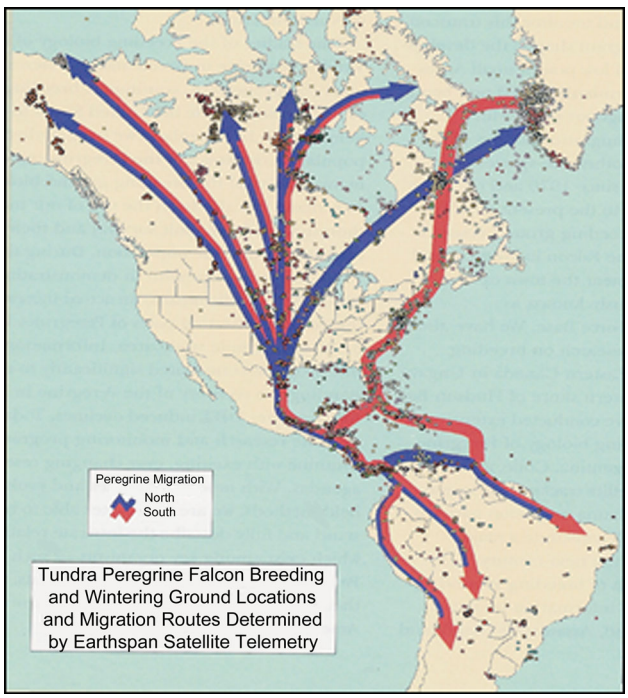

Fig. 1 Annual range and general migratory pathways of Tundra Peregrine Falcons determined by satellite telemetry

probing shorebirds are a monitoring priority due to direct exposure potential to residual DWH oil (OSAT 2011) and exposure through trophic processes due to PAH bioaccumulation in intertidal invertebrates (Meador et al. 1995; Peterson et al. 2003). External oiling of northern Gulf of Mexico shorebirds has recently been documented (Henkel et al. 2012). Consequently crude oil exposure in Peregrines could occur through direct contact with oil, preening of oiled feathers, and/or ingestion of oil when falcons capture and consume contaminated prey. Additionally, falcons may be more likely to capture avian prey compromised by crude oil exposure. Tundra Peregrines generally follow shorebird on migration and could therefore be exposed to DWH oil spill oil on or in prey throughout the annual cycle.

We report the spatial and temporal trends of circulating parent PAHs in migrant Peregrine Falcon blood collected from 2009 through 2011 on the Maryland and Texas Gulf Coasts, to assess crude oil exposure associated with the 2010 DWH spill.

Methods and materials

Capture and blood sampling

We captured and sampled blood from migratory Peregrines under existing survey protocols. Ward and Berry (1972), Hunt and Ward (1988) describe the study areas and capture techniques. Padre Island is located in the western-central Gulf of Mexico roughly 5–6 Peregrine migration days south of the DWH oil spill impact area for fall migrants, and is the only stopover/staging location visited by large numbers of spring migrants (Ward et al. 1978; Hunt and Ward 1988; Fuller et al. 1998; McGrady et al. 2002). Assateague Island is located on the Mid-Atlantic Seaboard, north of the DWH oil spill affected habitats for fall migrants, and was utilized as an unaffected reference area. Captured falcons were banded, visually inspected for oiled feathers, aged by plumage (juvenile <1 year, adult >1 year) and sexed by morphometric measurements according to the Bird Banding Lab, US Geological Survey criteria (Gustafson et al. 1997). A 2 ml blood sample was taken from the brachiocephalic vein with a heparinized 3 ml syringe/23 G needle (Redig 1993). Blood was transferred to vacutainers, kept in coolers while in the field and centrifuged at the end of the day. Plasma and red blood cells were separately drawn off by sterile pipette and transferred into cryovials, which were kept frozen until analysis (-20 °C).

Analytical chemistry

Peripheral blood cells were analyzed to quantify hematological levels of parent PAHs at The Center for Environmental Sciences and Engineering (CESE), University of Connecticut. We measured blood concentrations of the 16 parent PAH priority pollutants identified by the United States Environmental Protection Agency (USEPA 1989) using a Waters Acquity ultraperformance liquid chromatograph (Waters Corp.; Milford, MA) coupled to photodiode array, fluorescence, and tandem mass spectrometry (Paruk et al. 2014; Yeudakimaua et al. 2013). Which include; naphthalene, acenaphthylene, acenaphthene, flourene, phenanthrene, anthracene, fluoranthene, pyrene, crysene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a) pyrene, dibenz(a,h)anthracene, benzo(g, h, i)perylene, and Indenol(1, 2, 3-c-d)pyrene. Due to developments in DWH assessment for PAH sourcing, additional alkyl PAH analyses were conducted by CESE on the fall 2011 sample utilizing a Waters QuattroMicro gas chromatograph coupled to a tandem mass spectrometer (Waters Corp., Milford, MA) for 2-methyl naphthalene, 2,6-dimethyl naphthalene, 3-methyl phenanthrene, 9-methyl phenanthrene analytes. Laboratory reporting limits (RL) were 1.0 and 1.5 ng/g wet weight (ww) for parent and alky PAH analyses. All quality control data was within acceptable limits, the average data is presented in Table 1. There were no detectable levels of PAHs found in cryovial rinsate blanks.

CESE is a laboratory of record for the U.S Fish and Wildlife Service Natural Resource Damage Assessment (NRDA) for the DWH oil spill, and is currently part of a National Institute of Standards and Technology (NIST) intercomparison exercise for PAH metabolites in blood. By employing the same methods utilized in support of the Deepwater Horizon Shorebird NRDA, trophic level comparisons of PAH blood burdens will be possible when these data are made public.

Statistical methods

Our sampling design was not spatiotemporally "balanced" in the conventional sense, because we analyzed temporal replicates of blood samples from Fall 2009, Fall 2010, Spring 2011, and Fall 2011 South Padre migrations but only from the Fall 2010 Assateague migration. We therefore could not compare parameters and predictions from a model with interacting variables for island, year, and season because we did not have data from Assateague to match the other three migration periods at South Padre. We therefore pooled data into five migration groups representing each combination of island/year/season, and specified migration group as a categorical variable in PAH occurrence and concentration models. Non-detect samples, below the CESE upper reporting limit (1.5 ng/g ww), were treated as censored values and used in all statistical analysis. Summary statistics for total and individual PAH compounds were estimated using the Kaplan–Meier method with NADA (Nondetects and Data Analysis) library package for R (Helsel 2012). Differences in PAH distributions for

Table 1 Mean percent recoveries for QC standards across all batches of avian RBCs samples for Alkyl and Parent PAHs

	Blank	Duplicate RPD (%)	LCS Recovery (%)	Spike Recovery (%)	SRM Recovery (%)
Mean	BDL	7.6	89.2	74.0	79.4
SD	n/a	1.3	7.9	16.0	12.5
Acceptance range	<DL	80–120	80–120	50–125	50–125

RPD relative percent difference, *LCS* laboratory control sample, *SRM* standard reference material, *BDL* below detection limit, *DL* detection limit

seasonal, sex, and age cohort groupings were evaluated using Wilcoxon score tests followed by multiple pair-wise comparisons to assign statistical groupings ($p < 0.05$). False discovery rate control methods were used to control for family-wise type-1 errors in post hoc analysis ($\alpha_{FDR} = 0.05$). Kaplan–Meier graphs and statistical analysis was performed using JMP Pro10.0 statistical software.

Results and discussion

In preface

We captured and blood sampled 933 migrant Tundra Peregrines during 2009–2011 surveys and analyzed 189 samples among five migration cohorts (Table 2). We reasoned that monitoring circulating PAHs in peripheral blood cells is a relatively new technique (Perez et al. 2008), and therefore use a descriptive approach presenting our results. Percent occurrence or the frequency of PAH residue detection (n-PAH detects/n) and Kaplan–Meier estimates of mean PAH blood concentrations are descriptive metrics of the incidence or PAH distributions found in peregrine blood. No obvious plumage oiling was observed on Peregrines captured from 2010 to 2011. Seven parent PAH residues were detected throughout the study including; acenaphthene, fluorene, anthracene, fluoranthene, pyrene, benzo(a)pyrene, and dibenz (a,h)anthracene. One to seven PAH analytes were detected in each migration cohort, with a single compound (the primary PAH) contributing 58–100 % of the PAH profile. Parent PAH blood distributions illustrate age-class variability among 2010 fall migrants and in PAH analytes detected throughout the study period. The seasonal percent occurrence of detectable (>1.5 ng/g) PAH blood residues ranged from 6 % in spring migrants to 28–68 % in fall migrants (Table 1). Individual falcon ∑PAH blood concentrations ranged from <RL—43.5 ng/g and Kaplan–Meier seasonal mean ∑PAH blood concentration estimates from 1.70 to 11.49 ng/g, ww.

Temporal trends of PAHs

Peregrine PAH distributions in blood from 2010 Assateague and 2009 South Padre Island fall migrants are considered basal environmental levels (Table 2). Percent occurrence and mean ∑PAH blood concentrations were 28 %, 2.25 ± 0.19 ng/g and 48 %, 2.95 ± 0.42 ng/g respectively. The percent contribution of PAH residues of Assateague migrants were 58 % fluoranthene, 32 % anthracene, and 10 % dibenz(a,h)anthracene, the latter 2 compounds found only in juvenile falcons. The contributing PAH residues in 2009 South Padre Island migrants were 84 % fluoranthene, 11 % benzo(a)pyrene and 5 % anthracene. Benzo(a)pyrene was detected exclusively in juvenile falcons and anthracene only in adults. Seasonal ∑PAH distributions in peregrine blood were similar among basal study sites, according to Wilcoxon score tests and multiple pair-wise comparisons; as were those among basal sex and age cohorts (Table 2). Increased incidence of fluoranthene, the primary basal PAH, was found in South Padre migrants compared to those of Assateague Island (Table 3).

In 2010, 5 months after DWH oil spill, ∑PAH distributions increased significantly among fall South Padre Island migrants from basal cohort distributions, which was accompanied by a marked change in the PAH profile (Fig. 2). Seasonal percent occurrence of PAHs detected increased 20 % and mean ∑PAH blood residues (almost fourfold) to 68 % and 11.49 ± 1.24 ng/g respectively from 2009 Padre Island distributions. The percent contribution of PAH residues were 77 % fluorene, 10 % acenaphthene, 9 % pyrene, 2 % anthracene and 1 % each fluoranthene, benzo(a)pyrene and Dibenz(a,h)anthracene. PAH distributions varied with sex, age and PAHs detected, although male sample sizes were limited. The percent occurrence of detectable PAHs increased 27 % in female falcons. Juveniles (16.28 ± 1.25 ng/g) exhibited threefold higher blood concentration than adults (5.41 ± 1.10 ng/g), primarily the result of an increased incidence of relatively low molecular weight fluorene and marginal increases in acenaphthene. Pyrene incidence increased among adults (Table 3).

In 2011, seasonal ∑PAH distributions decreased to near basal levels among fall south Padre Island migrants (Fig. 2). Percent occurrence and mean ∑PAH blood residues were 42 % and 4.07 ± 0.30 ng/g respectively. An additional PAH profile shift was evident due to decreasing incidence of fluorene found in the 2010 cohort and increasing incidence of anthracene, which was similar by age

Table 2 Incidence and temporal distribution grouping ($p < 0.05$) of ∑PAHs (≥1.5 ng/g, wet weight) residues estimated in peripheral blood cells of migrant Peregrine Falcons captured at Assateague Island, Maryland, and South Padre Island, Texas, 2009–2011

Migration group[a]	Age/sex[b]	N	Frequency		∑PAH ng/g		Grouping[f]				
			Censored[c]	% Occur[d]	Mean ± SE[e]	Range	Sea	Age	Age	Sex	Sex
MD-F10	–	25	18	28	2.25 ± 0.19	1.8–5.4	A	–	–	–	–
	JV	17	13	24	2.61 ± 0.13	2.4–4.0	–	A	–	–	–
	AD	8	5	38	2.46 ± 0.53	1.8–5.4	–	–	A	–	–
	M	7	3	57	2.90 ± 0.28	2.4–4.0	–	–	–	A	–
	F	18	15	16	2.09 ± 0.26	1.8–5.4	–	–	–	–	AB
TX-F09	–	27	15	48	2.95 ± 0.42	1.5–8.6	A	–	–	–	–
	JV	15	9	40	2.70 ± 0.53	1.5–7.1	–	A	–	–	–
	AD	12	5	58	3.13 ± 0.73	1.8–8.6	–	–	A	–	–
	M	6	2	67	3.18 ± 1.02	1.5–7.1	–	–	–	A	–
	F	21	12	43	2.86 ± 0.47	1.8–8.6	–	–	–	–	A
TX-F10	–	71	23	68	11.49 ± 1.24	1.9–43.5	B	–	–	–	–
	JV	50	16	68	16.28 ± 1.25	8.9–43.5	–	B	–	–	–
	AD	21	7	67	5.41 ± 1.10	1.9–17.3	–	–	A	–	–
	M	2	2	0	<RL	<RL	–	–	–	A	–
	F	69	21	70	11.67 ± 1.26	1.9–43.5	–	–	–	–	C
TX-F11	–	33	19	42	4.07 ± 0.30	2.9–9.3	A	–	–	–	–
	JV	19	11	42	4.02 ± 0.42	2.9–9.3	–	A	–	–	–
	AD	14	8	43	5.09 ± 0.27	4.6–7.7	–	–	A	–	–
	M	9	4	56	4.43 ± 0.78	2.9–9.3	–	–	–	A	–
	F	24	15	38	4.99 ± 0.17	4.6–7.7	–	–	–	–	A
TX-S11	–	33	31	6	1.70 ± 0.00	1.7	C	–	–	–	–
	AD	33	31	6	1.70 ± 0.00	1.7	–	–	B	–	B

[a] Site (*MD* Assateague Island, *TX* South Padre Island)-season (*F* fall, *S* spring)/year

[b] *AD* adult (>1 year old), *JV* juvenile (<1 year old), *M* male, *F* female

[c] Censored/non-detects: samples below the adjusted (≥1.5 ng/g) laboratory lower limit of reportable concentrations (RL)

[d] % Occurrence-number of samples with PAH detected (≥1.5 ng/g) relative to number in cohort

[e] Summary statistics estimated using the Kaplan–Meier method with NADA (nondetects and data analysis) library package

[f] Season (sea), age, and sex groups not sharing a letter considered significantly different according to Wilcoxon score test and pairwise comparisons ($p < 0.05$) using an $\alpha_{FDR} = 0.05$

class (Table 3). An individual falcon captured and sampled during both fall 2010 and 2011 migrations illustrate this general trend; registering blood concentrations of 15.3 ng/g, and <RL of fluorene and anthracene respectively, as a juvenile fall migrant in 2010, then <RL and 5.0 ng/g of these compounds as a second year fall migrant in 2011. The percent contribution of PAH residues primarily consisted of anthracene, accounting for 80 % of the total PAHs detected. In addition, fluoranthene (11 %) was exclusively contributed by juveniles and acenaphthene (9 %) by adult falcons.

PAH sourcing

Sourcing and fingerprinting of PAHs in Peregrine blood are not possible due to the few parent PAH analytes detected (Neff et al. 2005; OSAT 2010; Tobiszewski and Namiesnik 2012). Fluoranthene, the most often detected PAH in basal blood burdens of fall migrants, suggests a pyrogenic source; associated with combustion of petroleum, wood and/or coal (Irwin et al. 1997; OSAT 2010). Interestingly, fluoranthene incidence decreased significantly in 2010 and 2011 fall South Padre Island migrants to below basal levels (Table 3). Increased avian blood concentrations of naphthalene, fluorene, anthracene, and pyrene resulted from recent exposure to weathered crude oil; a petrogenic PAH source (Perez et al. 2008). Our model-based seasonal comparisons of PAH analytes illustrate increasing incidence of fluorene and pyrene, with marginal increases in acenaphthene in 2010 fall South Padre Island migrants. As well, increased incidence of anthracene was detected among 2011 fall migrants (Table 3). In combination, the

Table 3 Incidence and temporal distributional grouping ($p < 0.05$) of PAH analytes (≥1.5 ng/g, wet weight) estimated in peripheral blood cells of migrant Peregrine Falcons captured at Assateague Island, Maryland and South Padre Island, Texas, 2009–2011

PAH	Migration group[a]	N	% Occur[b]	Mean PAH[c] ±SE (ng/g)	Range	Grouping[d]	
Acenaphthene	MD-F10	25	0	<RL	–	A	
	TX-F09	27	0	<RL	–	A	
	TX-F10	71	7	12.57 ± 0.11	12.4–17.4	A	
	TX-F11	33	3	6.70 ± 0.00	6.7	A	
	TX-S11	33	0	<RL	–	A	
Fluorene	MD-F10	25	0	<RL	–		A
	TX-F09	27	0	<RL	–		A
	TX-F10	71	48	13.05 ± 0.83	8.9–43.5		B
	TX-F11	33	0	<RL	–		A
	TX-S11	33	0	<RL	–		A
Anthracene	MD-F10	25	8	3.71 ± 0.02	3.7–4.0	A	
	TX-F09	27	4	2.70 ± 0.00	2.7	A	
	TX-F10	71	7	1.77 ± 0.05	1.7–4.2	A	
	TX-F11	33	33	4.99 ± 0.18	4.6–9.3	B	
	TX-S11	33	6	1.70 ± 0.00	1.7	A	
Fluoranthene	MD-F10	25	16	2.06 ± 0.18	1.8–5.4		B
	TX-F09	27	44	2.73 ± 0.33	1.8–8.6		A
	TX-F10	71	1	4.90 ± 0.00	4.9		BC
	TX-F11	33	6	2.99 ± 0.13	2.9–5.9		B
	TX-S11	33	0	<RL	–		C
Pyrene	MD-F10	25	0	<RL	–	A	
	TX-F09	27	0	<RL	–	A	
	TX-F10	71	15	2.54 ± 0.22	1.9–10.2	B	
	TX-F11	33	0	<RL	–	A	
	TX-S11	33	0	<RL	–	A	
Benzo(a)pyrene	MD-F10	25	0	<RL	–		A
	TX-F09	27	11	1.51 ± 0.01	1.5–1.8		A
	TX-F10	71	3	2.80 ± 0.01	2.8–3.0		A
	TX-F11	33	0	<RL	–		A
	TX-S11	33	0	<RL	–		A
Dibenz(a,h)anthracene	MD-F10	25	4	2.40 ± 0.00	2.4	A	
	TX-F09	27	0	<RL	–	A	
	TX-F10	71	4	2.24 ± 0.03	2.2–3.9	A	
	TX-F11	33	0	<RL	–	A	
	TX-S11	33	0	<RL	–	A	

[a] Site (*MD* Assateague Island, Maryland; *TX* South Padre Island, Texas)-season (*F* fall, *S* spring)/year

[b] Percent occurrence—number of samples with PAH detected (≥1.5 ng/g) relative to number in cohort

[c] Summary statistics estimated using the Kaplan–Meier method with NADA (nondetects and data analysis) library package

[d] Season (sea), age, and sex groups not sharing a letter considered significantly different according to Wilcoxon score test and multiple pairwise comparisons ($p < 0.05$) using an $\alpha_{FDR} = 0.05$

PAH profile changes, suggest one of a petrogenic PAH source after the DWH oil spill, from pyrogenic basal constituents found in Assateague and Padre Island migrants.

In addition, the alkyl PAH analysis of 2011 fall migrants also supports a petrogenic PAH origin (Table 4). Alkyl PAHs are more abundant in crude oil, more stable and persistent in the environment, and less prone to metabolization than parent PAH compounds (Irwin et al. 1997; OSAT 2010: Liu et al. 2012; Abers and Loughlin 2003). The parent compounds of naphthalene and phenanthrene were below the limit of detection, whereas alkyl homologues of these compounds were detected in 58 % of 2011 fall migrants with mean ∑alkyl concentration of 10.47 ± 1.96 ng/g

(Table 3). Thus potentially a reflection of the alkylated PAH content of DWH crude, weathered oil and oil mousse collected in salt marshes which were generally two to three times higher than that of associated parent PAH compounds (Liu et al. 2012).

PAHs among Peregrine age classes

The presence and persistence of PAH contaminants in Tundra Peregrine blood is not only a function of exposure duration but also the life cycle of red blood cells during erythropoiesis. The peak of PAH blood burdens in 2010 Padre Island autumnal migrants is attributed primarily to juvenile PAH exposure. The age-specific variation in PAH blood residues and profiles may be a result of the differing migration strategies and food habits among age classes. Berry (1971) and Hunt et al. (1975) maintain that the accelerated migration in adult Tundra Peregrines is a function of direct and efficient travel during migration. Survey mark-recapture efforts show that the minimum average stopover time (±95 % CI) of juvenile falcons (5.0 ± 0.5 days) is over two times longer than that of adults (1.9 ± 0.6 days) at South Padre Island. Our satellite tracking studies show that adult Tundra Peregrines on migration are likely to fly direct routes between breeding and wintering grounds. Upon arrival at the Gulf coast adult southbound migrants do not hesitate to cross the Gulf of Mexico, with little lateral movement along the coast. Juvenile Tundra Peregrines are known to engage in more exploratory behavior during migration, potentially arriving on the Gulf Coast close to or within the oil spill region and following the coastline southwest through Texas. Such falcons at Padre Island could have higher levels of petrogenic PAHs due to longer exposure to crude oil contamination or because of consuming more directly contaminated prey within the DWH oil spill vicinity. Additionally, juvenile Tundra Peregrines favor solitary prey as opposed to flocking birds (Ward and Laybourne 1985). Individuals of flocking prey species that are oil-compromised may be solitary and unable to proceed with the flock. If this results in being hunted and captured more often by juvenile Peregrines, this also potentially contributes to their higher PAH levels and differing compounds. This interpretation of results by age class is consistent with a route of

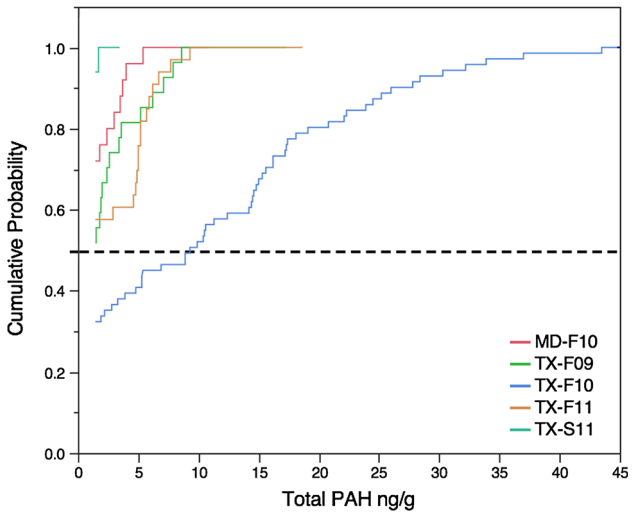

Fig. 2 Cumulative probability plots of the mean ∑PAH detected in Peregrine Falcon blood samples obtained on Assateague (MD) and Padre Islands (TX) from 2009 to 2011. Cohorts are defined as Site—season–year—site (MD, TX), season (*F* fall, *S* spring) year. The point of intersection between the Kaplan–Meier curve and dotted line indicate the median value for each group. Groups that do not have values below 0.5 are considered to have a median value below the level of detection

Table 4 Incidence of ∑alkyl PAHs and analytes (≥1.0 ng/g, wet weight) detected in peripheral blood cells of fall migrant Peregrine Falcons captured on South Padre Island, Texas, USA, 2011

Mig. Cohort[a]	Age/sex[b]	N	∑Alkyl PAH incidence				Alkyl PAH analyte detections (% Occ)			
			Cen[c]	% Occ[d]	Mean[e] (ng/g, ww.)	Range	2-methyl naphthalene	2,6-dimethyl naphthalene	3-methyl phenanthrene	9-methyl phenanthrene
TX-F11	–	33	14	58	10.47 ± 1.96	3.8–36.8	6 (18 %)	10 (30 %)	5 (15 %)	12 (36 %)
	JV	19	8	58	10.04 ± 2.54	3.8–36.0	3 (16 %)	6 (32 %)	3 (16 %)	9 (47 %)
	AD	14	6	57	11.86 ± 3.12	5.7–36.8	3 (21 %)	4 (29 %)	2 (14 %)	3 (21 %)
	M	9	3	67	5.9 ± 1.14	3.8–12.2	nd	nd	2 (22 %)	6 (67 %)
	F	24	11	54	13.05 ± 2.48	5.7–36.8	6 (25 %)	10 (42 %)	3 (13 %)	6 (25 %)

[a] Site-season–year—site-(TX) migration season (*F* fall, *S* spring), year

[b] Age class/sex—*AD* adult (>1 year old), *JV* juvenile (<1 year old), *M* male, *F* female

[c] Number of censored samples, below reporting limit of 1.0 ng/g

[d] % Occurrence—number of samples with PAH detected relative to number in cohort

[e] Mean blood cell concentrations, determined by Kaplan–Meier methods

contamination reflecting the geographic distribution of the DWH oil in the Gulf of Mexico.

Geographic patterns

The Tundra Peregrine is well-studied subspecies. Established knowledge of its migration dynamics and spring/fall migrant sampling greatly assists in directly relating these data to the geographic distribution of DWH oil in the northern Gulf of Mexico. Recall the roughly 30 day turnover of avian blood cells in vivo. Incidence of PAH residues in 2011 spring South Padre Island migrants were well below fall basal levels with a distinct migration cohort distribution (Table 2). Anthracene was the only PAH detected. The dramatic difference in PAH contamination of northward spring migrants demonstrates that the vicinity of South Padre Island and primary migration corridors to the south are relatively free of PAHs when compared with those to the north, as identified in southward fall migrants.

In summary

Seasonal blood distributions illustrate a peak in PAH exposure in fall 2010 South Padre Island migrants from basal levels, determined primarily in juvenile females. The PAH profile shifts suggest a change from a basal pyrogenic source to more petrogenic PAHs origins in 2010 and 2011. The exploratory migration and food habits of juvenile female peregrines, our most frequently captured falcons, suggests slower migratory passage than adults and likely greater utilization of northern Gulf of Mexico coastal habitats during migration. Combined spring and fall migration sampling, spatially limit significant changes in PAH exposure of Tundra Peregrines to the migratory corridors north of South Padre Island, where the DWH oil spill occurred.

Conclusions

Overall, our study suggests that fall Tundra Peregrine Falcon migrants were exposed to a pulse of crude oil contamination following DWH oil spill, by means of spatially and temporally tracking a corresponding PAH pulse in blood residues in this long-distance migrant. Due to the ubiquitous nature of PAHs in the environment, effective monitoring of crude oil exposure in avian species depends upon the development of ecosystem-based approaches in order to understand and ultimately predict chronic, delayed, and indirect long-term risks and impacts (Peterson et al. 2003). The National Research Council (NRC 2003) recommends establishing or expanding time-series monitoring of vulnerable ecosystem components likely to be exposed to petroleum releases. Efforts that include alkyl PAH analyses could improve understanding of crude oil exposure, related sex and age patterns and the annual variability of PAH exposure in Tundra Peregrines. This study demonstrates the utility of non-destructive blood monitoring techniques with an avian migrant. As well, we provide PAH reference values for future applications. The analysis of PAHs in Peregrine Falcon blood provides a convenient, repeatable method to monitor crude oil exposure in this avian predator.

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Ethical standards All field protocols were conducted under necessary permits acquired from state and federal authorities listed above.

Conflict of interest The authors declare that they have no conflict of interest.

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