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Novel and legacy per- and polyfluoroalkyl substances in bald eagle eggs from the Great Lakes region☆

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ABSTRACT

Decades of large-scale production of per- and polyfluoroalkyl substances (PFASs) have resulted in their ubiquitous presence in the environment worldwide. Similarly to other persistent and bioaccumulative organic contaminants, some PFASs, particularly the long-chain congeners, can be biomagnified via food webs, making top predators vulnerable to elevated PFAS exposure. In this study, we measured seven classes of PFASs in bald eagle (*Haliaeetus leucocephalus*) eggs for the first time. The eggs (n = 22) were collected from the North American Great Lakes in 2000–2012. The ranges of total concentrations of perfluoroalkyl sulfonic acids (∑PFSAs) and perfluoroalkyl carboxylic acids (∑PFCAs) were 30.5–1650 and 5.4–216 ng/g wet weight (ww), respectively. In addition to these traditional PFAS compounds, 6:2 fluorotelomer sulfonic acid (6:2 FTS; median: 15.7 ng/g ww), perfluoro-4-ethylcyclohexanesulfonic acid (PFECHS; 0.22 ng/g ww), and 8-chloro-perfluorooctanesulfonic acid (Cl-PFOS, detected in wildlife for the first time; 0.53 ng/g ww) were also frequently detected. Bald eagle eggs from breeding areas located less than 8 km from a Great Lake shoreline or tributary had significantly greater total PFAS concentrations (∑PFASs) than those from breeding areas located further than 8 km ($p < 0.05$). In these samples, ∑PFASs rivalled the total concentration of brominated flame retardants, and were significantly greater than those of several other organic contaminants, such as dechlorane-related compounds, organophosphate esters, and flame retardant metabolites.

1. Introduction

Per- and polyfluoroalkyl substances (PFASs) are a large group of synthetic compounds with strong carbon-fluorine bonds. PFASs have been produced since the 1950s, and they have been extensively used as protective coatings in a variety of commercial products and as additives in fire-fighting foams (Lopez-Antia et al., 2019; Singh et al., 2019). According to a recent report released by the Organization for Economic Co-operation and Development (OECD), over 4000 PFAS compounds are currently registered in the Chemical Abstracts Service (CAS) for the global market (OECD, 2018). Several decades of large-scale PFAS production has resulted in their ubiquitous distribution in various environmental compartments, even in remote areas (Wang et al., 2015). Due to the concerns over adverse toxicological effects and bioaccumulative potentials of medium- and long-chain PFASs (C_6 – C_{12}), the 3M Company voluntarily discontinued manufacturing the C_6, C_8, and C_{10} PFAS products in 2000, replacing them with C_4 PFAS (Buck et al., 2011). Perfluorooctanesulfonic acid (PFOS) and its salts were listed under Annex B of the Stockholm Convention in 2009, and perfluorooctanoic acid (PFOA) was also added to the Annex A of Stockholm Convention this year, with some exemptions (Jian et al., 2017; International Institute for Sustainable Development, 2019).

Bald eagles (*Haliaeetus leucocephalus*), occupying the top trophic level, are an ideal sentinel species for biomonitoring the levels of environmental contaminants, and in particular, organohalogen compounds (McKinney et al., 2006). Due to high exposure to persistent organic pollutants (POPs), the bald eagles' population declined during the 1950s–1960s. Following the phase-out of some notorious POPs (e.g. dichlorodiphenyltrichloroethane or DDT and polychlorinated biphenyls or PCBs), bald eagles started to recover in

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the mid-1990s (Route et al., 2014). Since some PFASs, especially long-chain congeners, can bioaccumulate and biomagnify (Fang et al., 2014) and are toxic for avian species (Peden-Adams et al., 2009; Nordén et al., 2016; Briels et al., 2018), it is urgent and important to assess PFAS exposure in bald eagles, high trophic level consumers. PFASs have been detected in gulls and cormorants from the Great Lakes (De Silva et al., 2016; Su et al., 2017), but there are only three reports about bald eagles (Kannan et al., 2001a; Kannan et al., 2005; Route et al., 2014), and none of them measured PFAS concentrations in their eggs. Previous studies have successfully utilized bird eggs as a less-invasive biomonitoring method to track avian exposure to PFASs in various regions (Lasters et al., 2019). Moreover, bird eggs can reflect maternal transfer of environmental pollutants, and are directly linked to hatching success (Gebbink and Letcher, 2012; Groffen et al., 2019; Lopez-Antia et al., 2019). Also, previous studies on PFASs in top-predators focused only on medium-to long-chain perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs). Given the market shift to alternative PFASs including short chain PFCAs, it is important to update the assessment to include newer compounds. In this study, in addition to medium and long chain PFCAs and PFSAs, we also targeted newer fluorinated compounds including fluoroalkyl sulfonamides (FSAs), fluoroalkyl sulfonamidoethanols (FSEs), telomer acids (FTAs), telomer alcohols (FTOHs), telomer sulfonic acids (FTSs), as well as short-chain PFCAs and PFSAs, for a total of 37 individual compounds. The objectives of the present study were to evaluate PFAS accumulation in bald eagle eggs from the Laurentian Great Lakes, as well as to investigate possible spatial patterns of concentrations in relationship to breeding area.

2. Materials and methods

2.1. Chemicals

The complete list of native and isotopically-labelled PFAS analytes is provided in Tables S1 and S2. The reference standards of target PFAS compounds, including 13 PFCAs, 10 PFSAs, 3 FSAs, 2 FSEs, 3 FTAs, 3 FTOHs, and 3 FTSs, as well as the mass-labelled PFASs used as the reference surrogate and internal standards were purchased from Wellington Laboratories (Guelph, ON, Canada). All individual standards had a purity ≥98%. Oasis WAX solid-phase extraction (SPE) cartridge (3 mL, 60 mg, 30 μm) and Envi-Carb (graphitized non-porous carbon) were obtained from Waters (Milford, MA) and Sigma-Aldrich (St Louis, MO), respectively. Centrifuge filters (nylon membrane, 0.2 μm) were purchased from VWR International (Radnor, PA). Water, methanol, isopropanol, and acetonitrile were all HPLC grade or higher. These solvents and formic acid, ammonium acetate, potassium hydroxide (KOH), Ottawa sand, and ammonium hydroxide were purchased from Fisher Scientific (Hanover Park, IL).

2.2. Sample collection and preparation

Non-hatched egg samples were provided by the Michigan Bald Eagle Biosentinel Program archive. Twenty-two eggs were collected in Michigan between 2000 and 2012. Egg sampling was conducted in compliance with the Animal Use Protocols of Clemson University (30067 & AUP2009-005) and of the University of Maryland (744587-2), as well as with the U.S. Geological Survey (USGS) Bird Banding permit and scientific collecting permits of the U.S. Fish and Wildlife Services (USFWS) and the Michigan Department of Natural Resources. The eggs were then transferred to the laboratory, cleansed of attached nest debris, and opened with a hacksaw blade (Best et al., 2010), stored at −20 °C and shipped to Indiana University for analysis. These samples were previously used in our laboratory for the analysis of other target chemicals (Guo et al., 2018; Stubbings et al., 2018). Based on the distance of the nest locations from a Great Lakes shoreline or a Great Lakes tributary where anadromous fish were accessible, egg samples were divided between inland (>8.0 km) and Great Lakes (<8.0 km) breeding areas (Fig. 1). The eggs from Great Lakes breeding areas were further classified according to their sampling locations between Upper and Lower Peninsulas of Michigan (Fig. 1). The bald eagle eggs were stored frozen at −20 °C in precleaned glass jar containers and sealed thoroughly. As ionic PFASs may adhere to glass (USEPA, 2017), their concentrations reported here might be

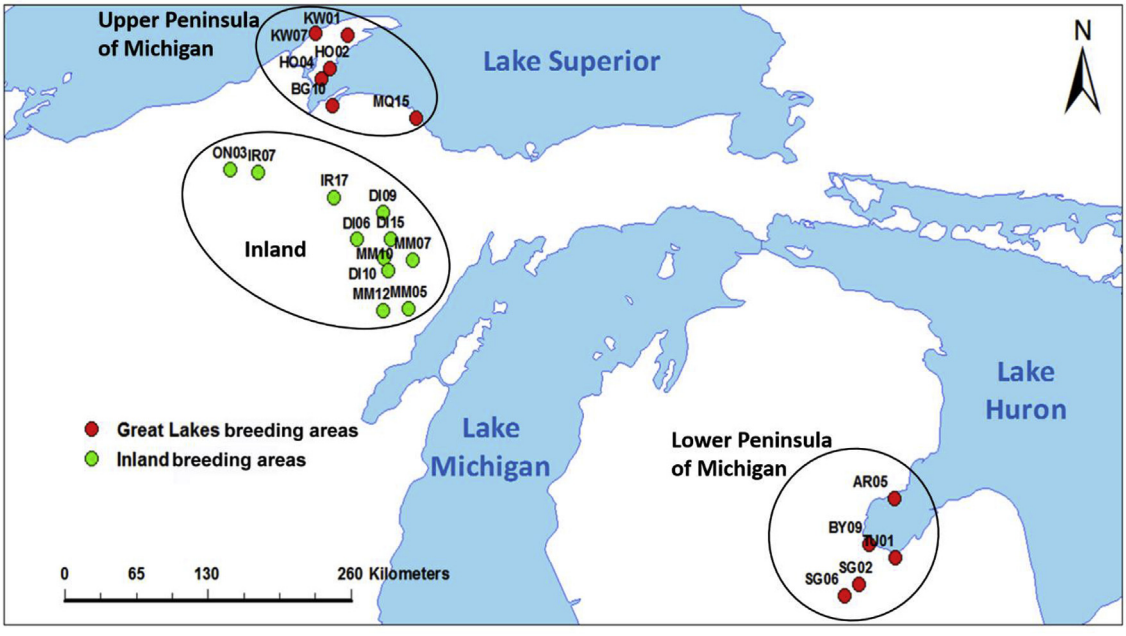

Fig. 1. Map of egg sampling sites and breeding area populations in the Great Lakes region.

underestimated. Before an aliquot of the egg was taken the sample was thawed in the refrigerator overnight and stirred for thorough homogenization.

2.3. Extraction and cleanup

The samples analyzed in this study were aliquots of the same egg homogenates our lab previously used for measuring other organic contaminants including brominated flame retardants (BFRs), dechlorane-related compounds (DECs), organophosphate esters (OPEs), and FR metabolites (FR-Ms) (Guo et al., 2018; Stubbings et al., 2018).

Measurements of PFASs followed previously reported analytical procedures with minor modifications (Chu and Letcher, 2008; Gewurtz et al., 2018). Briefly, 1 g of raw egg homogenate was spiked with 20 ng each of surrogate standards and subjected to extraction thrice using 3 mL of 10 mM potassium hydroxide (KOH) in acetonitrile/water (80/20, v/v). The volume of the combined extracts was reduced to 2 mL under nitrogen. The concentrated extract was diluted to 10 mL with water, adjusted to pH 4 with 2% formic acid (FA), and treated using an Oasis WAX (weak-anion exchange) cartridge. The WAX cartridges were firstly preconditioned with 1% ammonia in methanol (1% AM), MeOH, and water (6 mL each). Then, the sample passed through the cartridge at 1–2 drops per second under vacuum. The cartridge was washed with 1 mL of 2% FA followed by 2 mL water. The neutral PFASs were eluted with 3 mL MeOH/isopropanol (70/30, v/v, F1), while the ionic PFAS analytes were subsequently eluted by 3 mL of 1% AM (F2). The latter fraction was dried under gentle nitrogen (to remove ammonium hydroxide), re-dissolved in MeOH, and transferred to the tube holding F1. The combined eluate was diluted to 10 mL with MeOH and mixed with 100 mg of ENVI-Carb (graphitized non-porous carbon) by vortexing for 1 min. The sample was then centrifuged at 3000 rpm (850×*g*) for 5 min. Eight milliliters of supernatant were taken and concentrated to 0.5 mL under nitrogen. The resulting extract was filtered with a centrifugal filter (nylon membrane, 0.2 μm), and the filtrate was transferred to a 1-mL polypropylene (PP) vial. Internal standards (50 ng each) were added to the sample prior to instrumental analyses.

2.4. Instrumental analysis

An ultra-performance liquid chromatography coupled to triple-quadrupole mass spectrometer (Agilent 1290 Infinity II UPLC – 6470 QQQ-MS) was used to analyze ionic PFASs (i.e., PFCAs, PFSAs, FTAs, and FTSs) and FSAs. The LC/MS/MS was conducted under negative electrospray ionization mode (ESI^-). The compound separation on LC was achieved by an Acquity BEH C18 column (50 × 2.1 mm, 1.7 μm thickness, Waters, Milford, MA) at a column temperature of 40 °C. The mobile phases were 2 mM ammonium acetate (NH_4Ac) in water (A) and 2 mM NH_4Ac in MeOH (B). The initial phase B% was 10% and held for 0.5 min. Then it ramped to 40% in 0.5 min, and finally increased to 100% in 16.5 min. The instrument was equilibrated for 3.5 min between each run. The nebulizer, gas flow, gas temperature, capillary voltage, sheath gas temperature and sheath gas flow were set as 25 psi, 10 L/min, 300 °C, 2800 V, 330 °C and 11 L/min, respectively. The injection volume was 5 μL. MS was operated under multiple reaction monitoring (MRM) mode. The precursor and product ions of individual PFAS analytes were given in Table S1.

FTOHs and FSEs were analyzed on a gas chromatographic MS with positive chemical ionization (Agilent 7890B GC – 5977B PCI-MS), in the selected ion monitoring (SIM) mode. The SIM ions of individual GC analytes were presented in Table S2. A CP-Wax 57 CB column (25 m, 0.25 mm i.d., 0.20 μm thickness, Agilent, Santa Clara, CA) was used for the GC chromatographic separation. The injector was operated in the pulsed splitless mode and maintained at 200 °C. An injection volume of 1.5 μL was applied. The auxiliary port (AUX) temperature and carrier gas flow were set as 200 °C and 1 mL/min, respectively. The oven temperature was initially held at 60 °C for 3 min, increased to 85 °C in 10 min, and finally ramped to 190 °C in 7 min (held for 8 min).

2.5. Quality assurance and control

A matrix spike and a procedural blank consisting of 1 g Ottawa sand, previously muffled at 500 °C for 12 h, were processed along with each batch of 7–8 egg samples. A quantifier and a qualifier ion were monitored for each analyte. The identification and quantitation were based on three criteria: (1) sample peaks approximate Gaussian shapes; (2) retention times match those of reference standards within ±0.15 min; and (3) peak area ratios between two monitored ions/transitions are within 20% of theoretical values. A 6-point calibration curve was prepared over a concentration range of 1–200 ng/mL. The regression coefficients of calibration curves were all >0.99. Only one MRM transition with sufficient instrumental response was found for PFBA (Table S1); to overcome this problem, we included two mass-labelled PFBA standards, namely M3PFBA and MPFBA as surrogate and internal standards, respectively, for PFBA quantitation. The satisfactory recoveries of M3PFBA and relatively stable peak areas of MPFBA across our samples suggest that our experimental procedure resulted in negligible matrix effect on PFBA. The recoveries (average ± standard deviation) of surrogate standards were 89 ± 8%, 102 ± 11%, 83 ± 19%, 90 ± 5%, 94 ± 6%, 86 ± 18%, 118 ± 29%, 112 ± 27%, 86 ± 5%, 54 ± 8%, 77 ± 19%, 47 ± 12%, and 84 ± 14% for M3PFBA, M3PFBS, MPFHxA, MPFHxS, MPFOA, MPFOS, MFOEA, M2-8:2 FTS, MPFUnDA, dMeFOSA, M2PFTeDA, M2FOET, and dMeFOSE, respectively. Samples were corrected for recovery using the appropriate surrogate standards (see Table S3 for a list of surrogate and internal standards used for SS-correction and quantitation of individual PFAS analytes). Using this correction, recoveries were 87–129%. Data reported in this study were also blank corrected on a mass basis. PFAS residues in the procedural blanks are shown in Table S4. The method detection limits (MDL), ranging from 0.001 ng/g for PFBS to 1.4 ng/g for FDET (see Table S4), were defined as the minimum amount of analyte generating a peak with a signal-to-noise (S/N) of 5. PFAS concentrations were reported on wet weight basis (ng/g ww), unless otherwise stated.

2.6. Data analysis

OriginPro 2017 (OriginLab Corporation) and SPSS 20 (IBM Corporation) were used for data analysis and plots. Cells with values below MDLs were left empty for calculating summary statistics but they were replaced with MDL/2 for statistical analyses (i.e. correlation and spatial trends, restricted only to PFASs detected in ≥50% of the samples). All data were logarithmically transformed to assure normality and homogenous variance across groups, which were verified by the Shapiro-Wilk and the Brown-Forsythe tests, respectively. Correlation between variables were evaluated using Kendall's tau test or Hierarchical clustering analysis. Spatial variation in PFAS concentrations among Upper Great Lakes, Lower Great Lakes and Inland, were evaluated using the analysis of variance (ANOVA) with the Tukey's post-hoc comparison. The level of significance was set at $p = 0.05$. The octanol-water partitioning coefficients ($\log K_{OW}$) were taken from the U.S. Environmental Protection Agency Estimation Program Interface Suite Version 4.11 (see Table S4).

3. Results and discussion

3.1. PFAS profiles in bald eagle eggs

Concentrations of individual compounds measured in each sample are reported in Table S5 and a summary of the data is presented in Table 1.

PFASs were detected in all the egg samples with total PFAS concentrations (∑PFASs) in the range of 38.5–1800 ng/g, despite the absence of a documented PFAS contamination site within 20 km of any sampled breeding area (Environmental Working Group, 2019). These concentrations are greater than the levels detected in eggs of Great Lakes herring gulls (lower than bald eagles in the trophic level) collected during 2012–2013 (Letcher et al., 2015), and in peregrine falcon eggs (*Falco peregrinus*), another apex avian predator, collected from Sweden (2006), Norway (1991–2005), and South Greenland (2000–2014) (Gjershaug et al., 2008; Holmström et al., 2010; Vorkamp et al., 2019), but similar to those reported for white-tailed eagle (*Haliaeetus albicilla*) eggs from the Baltic Sea (2000–2014) and Norway (1991–2005) (Gjershaug et al., 2008; Faxneld et al., 2016) – see Table 2 for compound specific comparisons. Even higher PFAS concentrations would be expected in samples collected near identified PFAS pollution source(s) (Gewurtz et al., 2018).

PFOS, at a median concentration of 106 ng/g, was the most abundant among the target compounds, accounting for 63.3% of ∑PFASs (Table 1). Its burdens in biota may result from both direct exposure and precursor degradation (Dassuncao et al., 2017; Sedlak et al., 2017). PFOS has been consistently reported as the dominant PFAS in eggs of various bird species, e.g., herring gulls, cormorants, European starling, peregrine falcon, and white-tailed sea eagles from around the world (Table 2) (Letcher et al., 2015; Faxneld et al., 2016; Sedlak et al., 2017; Gewurtz et al., 2018). Despite its phase-out in the early 2000s by the 3M Corporation, PFOS remains abundant and ubiquitous due to its large historical production and ongoing releases from consumer products either in use or during disposal at landfill (Gewurtz et al., 2016). Bald eagle tissue (e.g. liver, kidney and muscle) from the Upper Peninsula of Michigan had a median PFOS concentration at 79.2 ng/g ww (Kannan et al., 2005). Other frequently detected PFSAs in the present study included PFDS, PFHxS, PFHpS, and PFNS. In contrast, short-chain PFSAs (i.e., PFPrS, PFBS and PFPeS), which are substitutes for the long-chain PFSAs, were hardly detected in our samples, suggesting lower bioavailability, shorter usage history, and lower capacity of *in ovo* transfer, relative to the legacy PFASs. PFBS was not observed in peregrine falcon eggs collected from the South Greenland (1986–2014) either (Singh et al., 2019; Vorkamp et al., 2019).

In addition to these PFSAs, we targeted other fluorinated sulfonic acids which have been less studied, especially in biota. Among these compounds, 1H,1H,2H,2H-perfluorooctane sulfonic acid (6:2 FTS), perfluoro-4-ethylcyclo-hexanesulfonic acid (PFECHS) and 8-chloroperfluoro-1-octanesulfonic acid (Cl-PFOS) were prevalent in these bald eagle eggs with detection frequencies ≥ 90% and concentrations up to 528, 21.9 and 3.46 ng/g, respectively.

In fact, 6:2 FTS, contributing to 14.2% of ∑PFASs, was the second most abundant PFAS detected. This compound is the main PFOS replacement in aqueous film forming foam and the predominant degradation intermediate of two major aqueous film forming foam constituents, i.e., 6:2 fluorotelomer sulfonamidoalkyl betaine (6:2 FTAB) and 6:2 fluorotelomermercapto-alkylamido sulfonate (6:2 FTSAS) (Weiner et al., 2013; Shaw et al., 2019). 6:2 FTS has been widely-detected in various media including indoor dust (Eriksson and Kärrman, 2015), water (Kaboré et al., 2018), and sewage sludge (Ruan et al., 2015). 6:2 FTS was assumed to be less persistent than the legacy PFASs that it has been substituting-namely PFOS-but it can degrade to short-chain perfluoroalkyl carboxylic acids, or to smaller fluorotelomer sulfonic acids via CF_2 "flake off" (Yang et al., 2014). Microbial biotransformation and oxidation processes in a UV/H_2O_2 system degrade 6:2 FTS into PFBA, PFPeA, and PFHxA rapidly (Yang et al., 2014; Shaw et al., 2019). Aerobic biotransformation of 6:2 FTS into PFPeA and PFHxA in activated sludge of waste water treatment plants was also observed by Wang et al. (2011). This degradation pattern is consistent with the clusters formed across 6:2 FTS, 4:2 FTS, and C_4–C_6 PFCAs in the dendrogram analysis (Fig. S1). Aside from this study, only Eriksson et al. reported 6:2 FTS levels of up to 52 ng/g in osprey eggs from Sweden (1997–2013) (Eriksson et al., 2016).

PFECHS, the major component in the 3M product marketed as FC-98, had an estimated production volume of 4.5–227 tons in the late 1990s (Howard and Muir, 2010). In the Great Lakes surface water, PFECHS levels were comparable to those of PFOS (De Silva et al., 2011), with concentrations ranging between non-detectable and 3.15 ng/g in Great Lakes herring gull eggs (2013–2014). These levels are lower than those in bald eagle eggs from this study (0.04–21.9 ng/g) (Letcher et al., 2015; Su et al., 2017).

Though Cl-PFOS was observed in a firefighter's serum sample during a non-targeted analysis (Rotander et al., 2015), data on its environmental occurrence remain extremely scarce, particularly in biological matrixes. A recent study found Cl-PFOS consistently below the detection limit in various terrestrial organisms in Oslo, Norway (Norwegian Institute for Air Research, 2017). The significant correlations between Cl-PFOS with other C_6–C_{10} PFSAs shown in the dendrogram in Fig. S1 (all $p < 0.05$) suggest that the Cl-PFOS

Table 1
Detection frequencies (DF, %), medians (MED, ng/g ww), concentration range (ng/g ww), average contribution to total (AC, %), of detectable PFAS analytes in the bald eagle eggs (n = 22).

	DF	MED	Range	AC
Ionic PFAS				
PFBA	59	0.09	<MDL[a] – 1.17	0.11
PFPeA	95	0.15	<MDL – 0.43	0.11
PFHxA	64	0.02	<MDL – 0.62	0.03
PFHpA	95	0.03	<MDL – 0.11	0.03
PFOA	82	0.04	<MDL – 0.26	0.02
PFNA	100	1.90	0.14–14.9	1.00
PFDA	100	4.56	0.71–42.0	3.11
PFUnDA	100	10.3	2.26–107	5.47
PFDoDA	100	2.01	0.27–26.3	1.13
PFTrDA	100	4.46	0.08–34.9	2.50
PFTeDA	100	1.80	0.26–11.3	0.87
PFHxDA	27	0.12	<MDL – 0.26	0.01
PFBS	27	0.01	<MDL – 0.04	0.01
PFHxS	100	1.35	0.02–10.7	0.95
PFHpS	100	0.66	0.17–3.50	0.38
PFECHS	100	0.22	0.04–21.9	0.28
PFOS	100	106	28.5–1338	63.3
PFNS	91	0.18	<MDL – 5.47	0.11
PFDS	100	6.32	0.29–295	5.33
Cl-PFOS	100	0.53	0.02–3.46	0.42
4:2 FTS	55	0.25	<MDL – 1.95	0.14
6:2 FTS	91	15.7	<MDL – 528	14.2
8:2 FTS	82	0.10	<MDL – 2.52	0.05
FHEA	5	5.58	<MDL – 5.58	0.14
∑Ionic-PFASs	***100***	***174***	***38.5–1804***	***99.7***
Neutral PFAS				
FOSA	95	0.05	<MDL – 1.97	0.03
MeFOSA	9	2.38	<MDL – 4.68	0.27
FOET	5	1.15	<MDL – 1.15	0.01
MeFOSE	5	0.17	<MDL – 0.17	0.00
EtFOSE	5	0.12	<MDL – 0.12	0.00
∑Neutral-PFASs	***100***	***0.06***	***0.01–4.68***	***0.31***
∑PFASs	***100***	***174***	***38.5–1805***	

[a] Below method detection limit (MDL).

Table 2
Summary of PFAS levels (ng/g or ng/mL wet weight) previously reported for top predator bird species (usually large birds of prey).

Continent	Country/Region	Bird Species	Sampling Year	Matrix (N[a])	PFSAs[b]	PFCAs[c]	Other PFASs	∑PFASs	Term	Ref.
America	Great Lakes Region	Bald eagle	2000–2012	Egg homogenate (N = 22)	C3[d] (0[e]); C4(0.01); C5(0); C6(1.4); C7(0.7); C8(106); C9(0.2); C10(6.3)	C4(0.1); C5(0.2); C6(0.02); C7(0.03); C8(0.04); C9(1.9); C10(4.6); C11(10); C12(2.0); C13(4.5); C14(1.8); C16(0.1)	PFECHS(0.2); Cl-PFOS(0.5); 4:2 FTS(0.3); 6:2 FTS(16); 8:2 FTS(0.1); FOSA(0.05); DF < 10%: GenX, FHEA, FOEA, FDEA, MeFOSA, EtFOSA, FHET, FOET, FDET, MeFOSE, and EtFOSE	174	Median	This study
America	Upper Midwestern U.S.	Bald eagle	2006–2015	Plasma (N = 381)	C8(335); C10(7.9)	C6(4.9); C7(0.18); C8(0.5); C9(3.7); C10(15)		113	Median	Elliott et al. (2019)
America	Upper Midwestern U.S.	Bald eagle	2006–2011	Plasma (N = 153)	C6(0.8–2.7); C7(1.1–2.6); C8(78–800); C10(4.0–265)	C4(0.3–0.6); C8(0.3–1.0); C9(4.2–8.1); C10(8.5–13); C11(7.8–18); C12(3.2–7.0); C13(2.2–3.9); C14(1.2–2.0)		163–941	Geomean range	Route et al. (2014)
America	South Carolina	Black/Turkey vulture, osprey, red hawk, and great horned owl	2009–2010	Liver (N = 16)	C6(N.G.[f]); C8(7.9–366);	C5–C14(N.G.); C16(N.G.)	FOSA(N.G.)	0–638	Concentration range	Yordy et al. (2013)
America	Great Lakes Region (Upper Peninsula of Michigan)	Bald eagle	2000	Liver (N = 6)	C6(0); C8(27–1740)	C8(0)	FOSA(0)		Concentration range	Kannan et al. (2005)
				Kidney (N = 4)	C6(0); C8(35–1480)	C8(0)	FOSA(0)			
				Muscle (N = 6)	C6(0); C8(0–96)	C8(0)	FOSA(0)			
				Testes (N = 1)	C6(0); C8(183)	C8(0)	FOSA(0)			
				Ovary (N = 1)	C6(0); C8(68)	C8(0)	FOSA(0)			
				Gall bladder (N = 1)	C6(0); C8(1490)	C8(0)	FOSA(0)			
America	Midwestern U.S.	Bald eagle	1990–1993	Plasma (N = 33)	C8(330)				Mean	Kannan et al. (2001b)
	OR, IL and CA, U.S.	Bald eagle	1995–1996	Liver (N = 4)	C8(192)					
	NC and OH, U.S.	Osprey	1996–1997	Liver (N = 3)	C8(52)					
Asia	Korea	Northern goshawk	2010–2011	Liver (N = 6)	C6(1.0); C8(710); C10(0.6)	C5(0); C6(0.20); C8(0.18); C9(1.7); C10(1.4); C11(3.7); C12(2.4); C13(2.3); C14(2.2)		728	Median	Barghi et al. (2018)
		Cinereous vulture		Liver (N = 7)	C6(0.2); C8(75); C10(0.2)	C5(0); C6(0); C8(0); C9(0.3); C10(0.6); C11(0.9); C12(0.6); C13(0.4); C14(0.3)		77		
		Common buzzard		Liver (N = 7)	C6(0.4); C8(97); C10(0.3)	C5(0); C6(0.1); C8(0.2); C9(1.0); C10(1.6); C11(3.8); C12(2.4); C13(3.1); C14(2.8)		133		
		Eagle owl		Liver (N = 5)	C6(1.1); C8(296); C10(1.0)	C5(0); C6(0); C8(0.2); C9(1.2); C10(5.2); C11(9.1); C12(8.4); C13(8.7); C14(5.3)		335		
Europe	South Greenland	Peregrine falcon	1986–2014	Egg homogenate (N = 41)	C4(0); C6(0.4); C7(0.9); C8(55); C10 (1.3)	C6(0); C7(0); C8(0); C9(3.0); C10(2.4); C11(7.4); C12(3.1); C13(1.4); C14(0.2)	FOSA(0)	77	Median	Vorkamp et al. (2019)
Europe	Norway	White-tailed eagle	2015–2016	Plasma (N = 70)	C6(0.1–1.6); C7(0–0.2); C8(5.3–17)	C8(0.1–0.5); C9(0.6–3.6); C10(0.4–1.4); C11(1.2–3.6); C12(0.3–0.6); C13(0.3–0.9)	FOSA(0–0.3); br[g]-PFOS(0.7–5.4)	9.2–32	Median range	Løseth et al. (2019)
				Feather (N = 70)	C6(0–3.3); C7(0–0.4); C8(0.2–1.7)	C6(0–0.3); C8(0–0.3); C9(0–0.4); C10(0–0.3); C11(0.2–0.8); C12(0.1–0.3); C13(0.3–1.1); C14(0–0.3)	FOSA(0.5–1.4)			
Europe	Norway	Northern goshawk	2014	Plasma (N = 10)	C6(0.7); C8(9.5)	C8(1.0); C9(1.4); C10(0.6); C11(1.8); C12(0.7); C13(1.0); C14(0.04)	br-PFOS(0.01); FOSA(0)	17	Median	Gómez-Ramírez et al. (2017)
		White-tailed eagle		Plasma (N = 14)	C6(0.6); C8(23)	C8(1.1); C9(4.1); C10(1.8); C11(4.0); C12(0.6); C13(1.2); C14(0)	br-PFOS(9.9); FOSA(0)	45		

(*continued on next page*)

Table 2 (*continued*)

Continent	Country/Region	Bird Species	Sampling Year	Matrix (N[a])	PFSAs[b]	PFCAs[c]	Other PFASs	∑PFASs	Term	Ref.
				Feather (N = 14)	C6(0.1); C8(4.9)	C8(0.3); C9(0.8); C10(0.4); C11(0.9); C12(0.3); C13(1.0); C14(0.01)	br-PFOS(1.5); FOSA(1.4)	12		
Europe	Norway	White-tailed eagle	2011–2012	Plasma (N = 35)	C8(34)	C9(2.3); C11(5.8)		42	Median	Sletten et al. (2016)
Europe	Baltic Sea	White-tailed eagle	1966–2010	Egg homogenate (N = 83)	C6(0.1–5.0); C8(6.9–1514); C10(0.01–11);	C8(0.04–2.2); C9(0.06–47); C10(0.02–41); C11(0.04–50); C12(0.02–11); C13(0.1–46); C14(0.02–4.1); C15(0.01–3.2)	FOSA(0.01–1.6)	7.8–1703	Geomean range	Faxneld et al. (2016)
Europe	Sweden	Osprey	1997–2001, 2007–2008, and 2013	Egg homogenate (N = 10)	C8(62–97)	C9(0.9–1.5); C10(3.2–7.6); C11(5.1–11); C12(2.0–5.3); C13(1.7–5.6); C14(0.2–0.9)	br-PFOS(2.0–6.0); 6:2 FTS(levels up to 52)		Median range	Eriksson et al. (2016)
Europe	Shetland, Scotland	Great skua	1980–2008	Egg homogenate (N = 39)	C6(0–0.1); C7(0.10–0.13); C8(19–23); C10(0–0.2)	C9(0–0.5); C10(0.1–1.5); C11(0.7–10); C12(0–2.9); C13(0.5–7.2); C14(0.03–0.6)	FOSA(0.05–0.09)		Median range	Leat et al. (2013)
				Plasma (N = 19)	C6(0.4–0.6); C7(0.14–0.20); C8(24–37); C10(0.3–0.4)	C7(0.01–0.04); C8(0.09–0.25); C9(0.7–1.1); C10(1.4–2.0); C11(9.1–14); C12(2.7–4.0); C13(8.7–15); C14(1.0–1.8)	FOSA(0.07–0.99)			
Europe	Norway	White-tailed eagle	2008–2010	Plasma (N = 36)	C8(25–39)			32–55	Mean range	Sonne et al. (2012) and Bustnes et al. (2013)
		Goshawk		Plasma (N = 56)	C8(12–22)			21–151		
		Golden eagle		Plasma (N = 12)				0.9–12		
Europe	Sweden	Peregrine falcon	2006	Egg homogenate (N = 10)	C6(0.8); C8(83); C10(0.7)	C9(1.6); C10(3.1); C11(4.2); C12(3.2); C13(7.3); C14(2.7); C15(0.6)		107	Mean	Holmström et al. (2010)
Europe	Baltic Sea	White-tailed eagle	1979–2000	Liver (N = 44)	C8(0–127)				Concentration range	Kannan et al. (2002)
Europe	Norway	White-tailed eagle	1991–2005	Egg homogenate (N = 75)				~150[h]	Median	Gjershaug et al. (2008)
		Golden eagle						~50		
		Peregrine falcon						~100		
Europe	West Greenland	White-tailed eagle	1984–2013	Feather (N = 31)	C8(3.3)	C8(0.7); C9(1.6); C10(0.3); C11(1.7); C12(0.3); C13(2.3)	FOSA(3.8)	13.4	Mean	Sun et al. (2019)
	Norway		1971–2015	Feather (N = 66)	C8(6.9)	C8(0.6); C9(0.4); C10(0.4); C11(1.0); C12(0.6); C13(2.1)	FOSA(2.2)	13.2		
	Sweden		1968–2011	Feather (N = 50)	C8(13)	C8(0.9); C9(1.3); C10(0.6); C11(1.2); C12(0.5); C13(1.2)	FOSA(3.3)	20.4		

[a] N = sample size.
[b] PFSAs = Perfluoroalkyl sulfonic acids.
[c] PFCAs = Perfluoroalkyl carboxylic acids.
[d] Length of carbon chain. For example, C8 in the column "PFSAs" refers to perfluorooctanoic sulfonic acid.
[e] "0" = not-detectable or below method detection limit.
[f] Detailed values were not given in the reference.
[g] br = branched.
[h] Values visually estimated from a plot.

burden in bald eagle eggs (0.02–3.46 ng/g ww) may arise from its presence as an impurity in medium- and long-chain PFSA technical mixtures. Another possible source of Cl-PFOS is octane sulfonyl chloride, an electrochemical fluorination raw material for production of perfluorooctanesulfonyl fluoride (POSF, widely used in the PFOS synthesis) (Pabon and Corpart, 2002).

Among the targeted PFCAs, PFUnDA, with a median concentration of 10.3 ng/g, was the predominant congener. Median concentrations of other longer chain PFCAs (C ≥ 9) ranged from 1.80 to 4.56 ng/g ww, while those for shorter chain PFCAs were below 1 ng/g ∑PFCA concentrations in bald eagle eggs were generally one order of magnitude lower than those of ∑PFSAs, which is similar to what was previously observed in bird eggs worldwide (Table 2) (Letcher et al., 2015; Sedlak et al., 2017; Vorkamp et al., 2019). GenX, a PFOA replacement, was also monitored, but it was not detected in any of our samples.

Detection frequencies for the remaining PFAS compounds, i.e., telomer alcohols and acids, fluoroalkyl sulfonamides and sulfonamidoethanols, were all ≤10%, except for FOSA. FOSA is an immediate PFOS precursor that was quantifiable in 95% of our egg samples, though at relatively modest levels (median: 0.05 ng/g). Restriction on FOSA applications in industrial processes from 2002 and possible biotransformation of FOSA into PFOS, have led to recent declines in its concentrations in the environment (Gebbink et al., 2009; Letcher et al., 2015). Significant associations ($p < 0.05$) between FOSA and C_7–C_{10} PFSAs suggest common sources for these compounds (Fig. S1). The absence of FTOHs and FSEs in these samples is likely due to *in vivo* metabolism converting them into PFCAs (Gebbink et al., 2011).

3.2. Spatial trends

Spatial trends of PFAS concentrations were evaluated using ANOVA. The Tukey's post hoc comparison revealed that Great Lakes eggs from Michigan's lower peninsula contained significantly greater ∑PFASs than those from Michigan's upper peninsula and higher than those in the inland samples (Fig. 2 and Table S6). A similar spatial trend was also found for various individual compounds including PFOA, PFOS and most of the long chain PFSAs and PFCAs, as well as 8:2 FTS (Table S6). Several possible PFAS sources are present in the Lower peninsula, including Wurtsmith Air Force Base (in Oscoda, MI) and Camp Grayling Army Airfield (in Grayling, MI) (Environmental Working Group, 2019).

Elevated bald eagle exposure to PFASs in the Great Lakes breeding areas could be attributed to the presence of contamination sources along the Great Lakes shoreline or upstream of Great Lakes tributaries or to closer proximity to more populated centers (Environmental Working Group, 2019), resulting in higher PFAS burdens in the prey fish these bald eagles feed on. Diet has been considered one of the most important routes for biota exposure to PFASs (Fang et al., 2014; Xu et al., 2014; Jian et al., 2017). This spatial trend is not unique to PFASs, though. Greater concentrations of polybrominated diphenyl ethers (PBDEs) and dechlorane-related compounds were observed by Guo et al., in these same samples (Guo et al., 2018), and higher levels of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) were detected in plasma of Great Lakes bald eagle nestlings (Bowerman et al., 2002).

The spatial trend for ionic PFASs was more pronounced for larger molecules (e.g. $C_{8\text{-}14}$ PFCAs, $C_{7\text{-}10}$ PFSAs, PFECHS, and Cl-PFOS, all $p < 0.05$) than for smaller ones, like $C_{4\text{-}6}$ PFCAs, PFHxS, 4:2 FTS, and 6:2 FTS (all $p > 0.05$). In general, larger molecules present higher values of $\log K_{OW}$, which negatively affects their ability to travel long distances. In contrast, no spatial patterns were detected for neutral PFAS compounds (mainly FOSA), likely due to their semi-volatile properties and higher potential to undergo substantial atmospheric transport (Xie et al., 2015).

4. Environmental and toxicological implications

In our previous studies we monitored several other categories of organic contaminants in the same bald eagle eggs (Guo et al., 2018; Stubbings et al., 2018). Despite the fact that the environmental fate and behavior and transport routes for other contaminants might be different than for PFASs, a comparison between these groups of

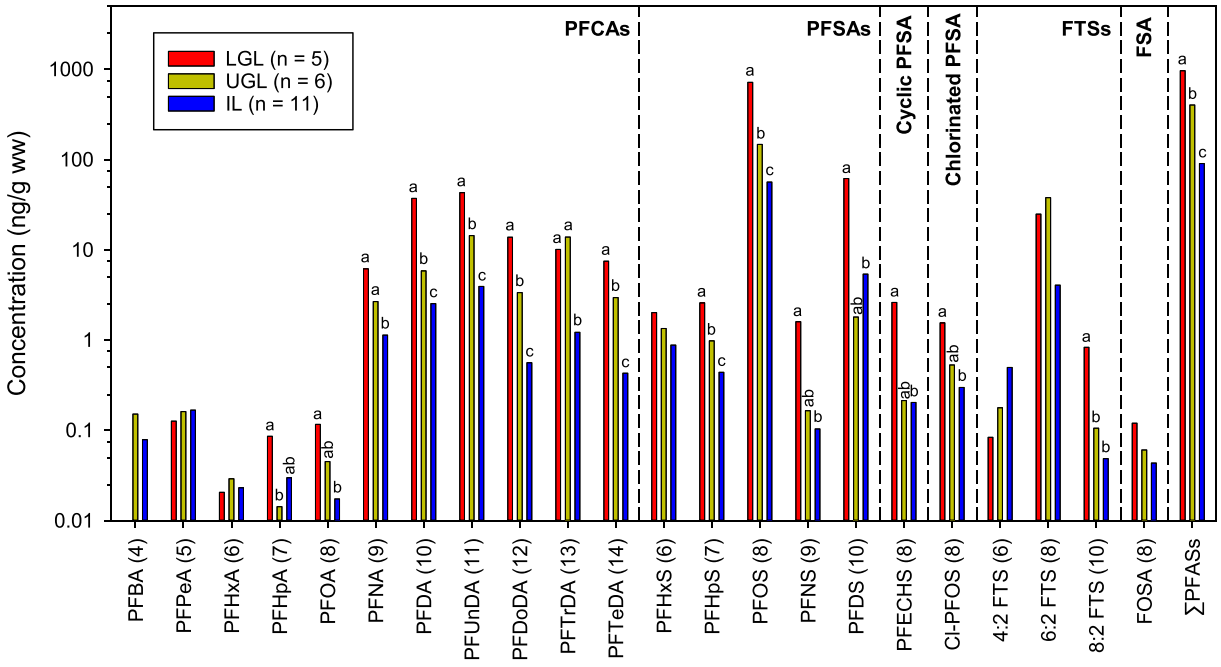

Fig. 2. Median concentration of PFASs with detection frequencies ≥50% in the bald eagle eggs from inland (IL), upper and lower Great Lakes (UGL and LGL). The number between brackets represents the number of carbon atoms. Bars without a common letter indicate a significant difference ($p < 0.05$) in PFAS concentrations between the three regions.

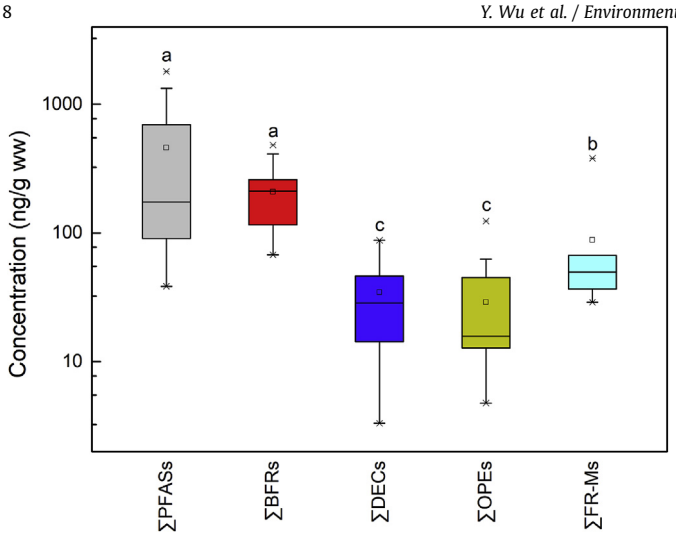

Fig. 3. Comparison of PFAS concentrations with those of other organic contaminants, including brominated flame retardants (BFRs) (Guo et al., 2018), dechlorane-related compounds (DECs) (Guo et al., 2018), organophosphate esters (OPEs) (Guo et al., 2018), and FR metabolites (FR-Ms) (Stubbings et al., 2018), in the bald eagle eggs collected from the Great Lakes region. Boxes sharing the same letter are not significantly different ($p > 0.05$).

compounds provide useful information.

In these eggs, the concentrations of ∑PFASs are significantly greater than those of DECs, OPEs, and FR-Ms (all $p < 0.001$, Fig. 3) and are similar to those of total BFRs. No significant associations between the concentrations of ∑PFASs and any of these compounds were found, confirming that these classes of compounds have different sources due to their diverse uses and manufacturing locations. Unlike other non-ionizable persistent organohalogen contaminants which are bioaccumulated majorly via dietary intake, ionic PFASs, accounting for over 99% of ∑PFASs in our samples, can also enter aquatic organisms (e.g. fish) through the gills and partition to other tissues via binding to serum proteins, enterohepatic circulations, and saturable renal resorption (Ng and Hungerbühler, 2013; Fang et al., 2014; De Silva et al., 2016).

Data on the toxicity of PFASs, particularly PFASs other than PFOS, to avian species remain limited. Field studies on PFAS exposure of tree swallows (*Tachycineta bicolor*) showed a negative correlation between PFOS burdens in eggs and hatching success, at concentrations of 150–200 ng/g ww (Custer et al., 2012; Custer et al., 2014). PFOS in chicken eggs (*Gallus gallus domesticus*) at environmentally relevant concentrations could lower embryonic heart rate, enhance oxidative stress, and cause immune alteration and brain asymmetry (Peden-Adams et al., 2009; Briels et al., 2018). *In ovo* studies using chicken eggs suggested that PFOS, PFHxS, and PFUnDA at concentrations of 100, 890, and 10,000 ng/g ww in the yolk, respectively, could cause adverse effects, including altered mRNA expression and T4 level disturbance (Letcher et al., 2015). In the present study, 55% of the bald eagle eggs (homogenate) contained PFOS above 100 ng/g. The estimated toxicity reference value and predicted no effect concentration of PFOS in egg yolk of trophic level IV fish-eating birds, including eagles, were 1.7 and 1.0 μg/mL, respectively (Newsted et al., 2005). Tartu et al. and Costantini et al. reported that exposure to long-chain PFCAs may induce higher protein oxidative damage, decreased baseline corticosterone, and lower hatching success for Arctic black-legged kittiwakes (*Rissa tridactyla*) (Tartu et al., 2014; Costantini et al., 2019). Due to mounting evidence of PFOS toxic effects, several organizations created quality guidelines and standards. For example, the Canadian environmental quality guideline of PFOS in bird eggs is 1.9 μg/g (Environment and Climate Change Canada, 2018), greater than the maximum PFOS level we observed (1.34 μg/g). A few recent field studies on tree swallows and great tits (*Parus major*) nesting in regions heavily polluted by PFASs showed no evidence that high PFAS exposure cause reproductive impairment (Custer et al., 2019; Groffen et al., 2019), which blurs the effects of PFASs on avian reproduction.

The data presented here are likely only the tip of the iceberg on the number of PFASs present in bald eagles. Non-target studies continually discover novel alternative PFASs synthesized to substitute for legacy PFASs (Fakouri Baygi et al., 2016; Yu et al., 2018), byproducts or impurities in legacy PFAS commercial products (similar to the Cl-PFOS measured in this study) (Rotander et al., 2015; Yu et al., 2018), and PFASs produced in high-volumes over a long time (for example, *p*-perfluorous nonenoxybenzenesulfonate) (Xu et al., 2017). Though several emerging PFASs have been monitored in our study, it is crucial to continue monitoring these compounds in all levels of the Great Lakes food chain, including fish and bald eagles, to identify new and emerging compounds as well as PFASs of greatest concern (e.g. highly persistent and bioaccumulative (Fakouri Baygi et al., 2016). Also, toxicity data on these compounds is virtually absent, as well as information on the possible additive or synergistic effects induced by multiple PFASs or by the co-presence of PFASs with other contaminants.

CRediT authorship contribution statement

Yan Wu: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft. **Kendall L. Simon:** Writing - review & editing, Conceptualization. **David A. Best:** Writing - review & editing, Conceptualization. **William Bowerman:** Writing - review & editing, Funding acquisition, Conceptualization. **Marta Venier:** Supervision, Project administration, Funding acquisition, Writing - review & editing.

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Appendix A. Supplementary data

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