



Bald eagles as bio-sentinels: Monitoring and fingerprinting PFAS contamination

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ABSTRACT

The present study assessed bald eagle (*Haliaeetus leucocephalus*) nestlings as a sentinel species for per- and polyfluoroalkyl substances (PFAS) fingerprinting tools due to their high trophic position, well-characterized life history, and restricted foraging ranges during nesting. From 2023 to 2025, we sampled 115 bald eagle nestlings across seven regions in Wisconsin, Lake Superior, and Lake Michigan to quantify plasma PFAS concentrations and evaluate regional contamination fingerprints. Total PFAS levels were highest in the Middle Wisconsin River and Apostle Islands, and lowest in the Upper Wisconsin River. Regional compositional profiles revealed distinct patterns: Middle Wisconsin River nestlings showed elevated PFOS and precursor compounds, while Apostle Islands nestlings exhibited elevated short chain and legacy compounds such as PFBS, PFNA and PFUnA. Principal component analyses and correlation structures further distinguished sites, suggesting both point-source contamination and diffuse inputs. These findings underscore the utility of bald eagles as biomonitors for spatially explicit PFAS contamination and demonstrate how fingerprinting approaches can improve source attribution and risk assessment in Great Lakes ecosystems.

1. Introduction

Per- and poly-fluorinated alkyl substances (PFAS) are a large group of man-made organofluorine chemicals that are used in numerous industrial and consumer applications (e.g., fabric weatherproofing and fire-fighting foams) (De Silva et al., 2021). PFAS can be released into the environment throughout their entire life cycle from production to disposal and are introduced through both point sources (e.g., landfills, manufacturing plants, industrial sewage treatment plants, or fire-fighting training areas) and nonpoint sources (e.g., wet and dry atmospheric deposition) (Crone et al., 2019; Koch et al., 2019; Podder et al., 2021). While most research and sampling efforts have focused on two specific PFAS, perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) (e.g., persistent organic pollutants), PFAS exposure in the environment most often occurs as complex mixtures (Podder et al., 2021). PFAS are found in surface waters all around the world (Ackerman Grunfeld et al., 2024), including the Great Lakes and

adjacent regional rivers (Xia et al., 2024). PFAS concentrations in the Great Lakes region vary widely depending on the matrix sampled – for example, water levels (<50 ng/L) (Remucal, 2019; Scott et al., 2010), sediment (<20 ng/g) (Remucal, 2019), fish (<140 ng/g) (Balgooyen et al., 2026; Point et al., 2021), and avian eggs (<400 ng/g) (Remucal, 2019; Wu et al., 2020). Because of this variation, it is essential to measure PFAS across multiple biological compartments, particularly in top predators, to develop a comprehensive understanding of ecosystem health.

Apex predators, like bald eagles (*Haliaeetus leucocephalus*), can be key sentinels of ecosystem health and contamination due to their high trophic position in the food web and their well-understood population, distribution, and reproductive status (Bowerman et al., 2002; Dykstra et al., 2010). Bald eagles are interconnected within aquatic ecosystems, where environmental pollution can concentrate at high levels (Colborn, 1991; Deblonde et al., 2011). Particularly for PFAS, exposure can occur through consumption of both terrestrial food sources (e.g., deer, turkey,

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etc.) and aquatic food sources (e.g., fish, turtles, etc.), as they are ubiquitous throughout the environment (Cartron et al., 2025; Gkika et al., 2025; Remucal, 2019; Witt et al., 2024). Thus, PFAS exposure can impact scavenger/generalist predators, especially with main food sources coming from aquatic environments. Because bald eagles are consumer-scavengers with varying diets, measuring contaminants in nestlings can allow for spatial comparisons of contaminant concentrations between locations and potentially identify unique PFAS fingerprints that could elucidate bioaccumulation factors and potentially different biomagnification patterns (Garrett et al., 1993; Route et al., 2014; Watson, 2002).

Individual Great Lakes, river systems, and other water bodies can have unique proportions of PFAS, likely based on a variety of reasons (e.g., contamination source, breakdown products, etc.). Because PFAS bioaccumulate and biomagnify in wildlife across aquatic and terrestrial food webs, they could provide a unique fingerprint of sources in a region (George et al., 2023). Moreover, PFAS are subject to physiological processes of absorption, distribution, metabolism, and excretion (ADME), which could alter their fingerprints in biota, from biotransformation, relative to their environmental sources (Lin et al., 2021). PFAS can exist in various ionic states, such as anions (acids), cations, or even zwitterions and because some PFAS are thought to be ‘proteinophilic’ (i.e., bind to proteins rather than lipids) (Houde et al., 2006; Kelly et al., 2009; Li et al., 2022; Ng and Hungerbuehler, 2015), which can play a role in ADME (Bangma et al., 2022), it is unclear how using biota for PFAS fingerprints could be used to identify PFAS sources. Understanding how PFAS fingerprints differ across abiotic and biotic matrices, and how they align with one another, could be used together to improve source identification.

Due to the ubiquitous nature and the potential toxicity of PFAS to wildlife, utilizing sentinel species for biomonitoring is an important tool to inform regulatory decisions, wildlife management, and conservation efforts (Dykstra et al., 2021; Strom et al., 2025). Therefore, the objective of this study was to evaluate current (2023–2025) concentrations of PFAS in bald eagle nestlings from seven different regions throughout Wisconsin, Lake Superior, and Lake Michigan, and identify contaminant

patterns or fingerprint signatures based on regions. Our results can be used by management agencies to highlight locations of concern that could be prioritized for remediation, identify relationships with specific PFAS to elucidate abiotic and biotic transformations for fingerprint signature mapping, inform potential regions with unique bioaccumulation patterns, and provide the bases for understanding PFAS impacts on wildlife health in the Great Lakes and Wisconsin river systems.

2. Materials and methods

2.1. Field collections

Bald eagles were handled and sampled under the USGS federal banding permit 05322 and University of Wisconsin – Madison IACUC protocol L006878-A01. Each year from mid-April through early June, aircraft were used to identify occupied and productive bald eagle nests (e.g., the presence of nestlings) from study locations across Wisconsin: Apostle Islands (AIS), Lake Superior South Shore (SSS), Fox River/Lower Green Bay (FRGB), Upper Green Bay/Lake Michigan (GBLM), Lower Wisconsin River (LWR), Middle Wisconsin River (MWR), and the Upper Wisconsin River (UPWR) (Fig. 1A; Table 1). While AIS and SSS are in proximity, they are significantly different since AIS is made up of a series of islands managed by the National Park Service as a national lake shore, while SSS is made up primarily of private landowners. When nestlings were 4 to 9 weeks old, the nest tree was climbed, and the eaglets were lowered to the ground for sampling. Biometric measurements were taken for each eaglet, including total weight (kg), footpad length (mm), hallux claw length (mm), bill depth (mm), culmen length (mm), and the length of the 8th primary feather (mm). All measurements followed methods previously described by others (Bortolotti, 1984; Bowerman et al., 2002; Dykstra et al., 2005). The age of the nestling was estimated by the length of their 8th primary feather (Bortolotti, 1984).

We collected approximately 11 ml of blood from the brachial vein of nestlings using a sterile 12 ml polypropylene syringe attached to an infusion set fitted with a 21-gauge needle. Approximately 10 ml of blood

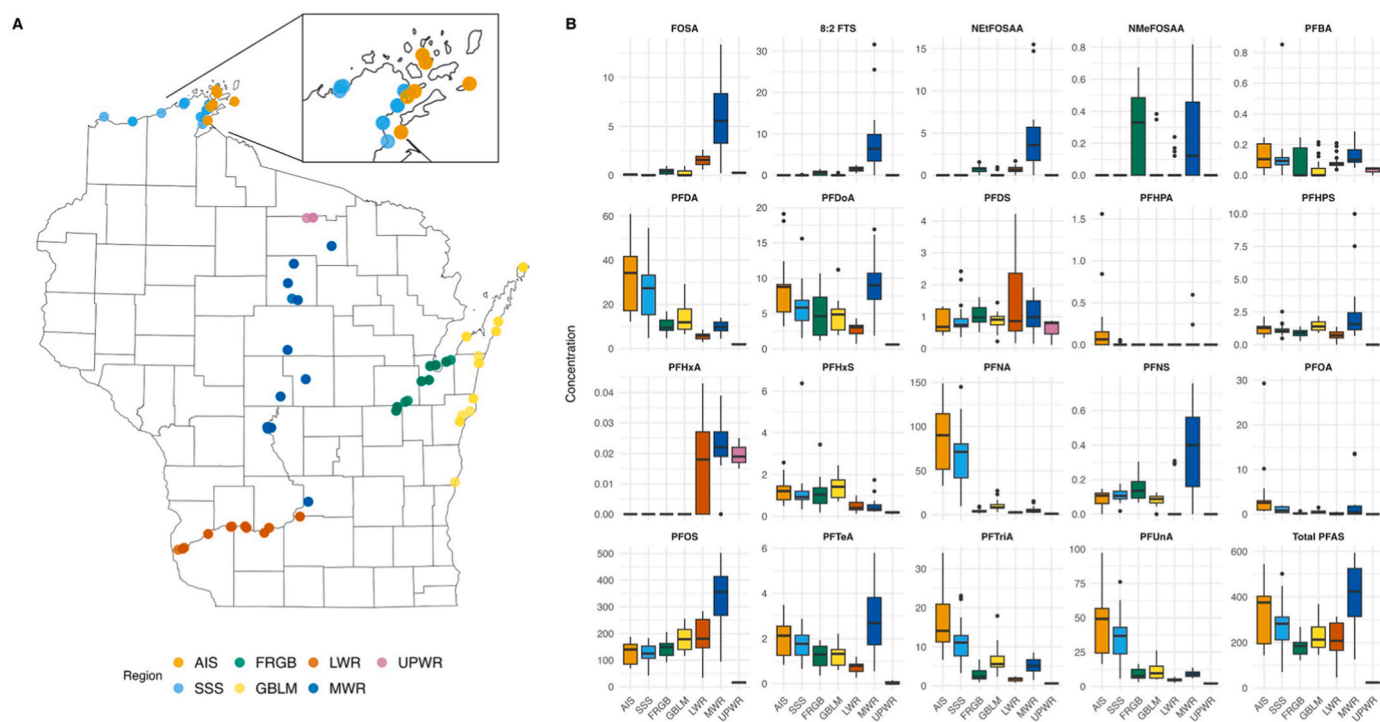


Fig. 1. (A) Sampling sites from 2023 to 2025 across the state of Wisconsin. Sites are colored according to geographic region. (B) Average values of PFAS analytes and total PFAS grouped by region (ng/ml = ppb).

Table 1

Blood sample sizes and years sampled for each of the sites. Sample size of females are in parentheses. Total sample size represents all the sites combined.

Sample year	Apostle Islands (AIS)	Fox River Green Bay (FRGB)	Green Bay/Lake Michigan (GBLM)	Lake Super South Shore (SSS)	Lower WI River (LWR)	Middle WI River (MWR)	Upper WI River (UPWR)	Total
2023	6 (3)	11 (5)		11 (7)				28 (15)
2024	9 (3)	6 (3)	19 (9)	6 (3)				40 (18)
2025					21 (5)	23 (9)	3 (2)	47 (16)
Total	15 (6)	17 (8)	19 (9)	17 (10)	21 (5)	23 (9)	3 (2)	115 (49)

was immediately transferred to a sterile sodium heparin-containing vacutainer, while the remaining 1 ml was transferred to a sterile vacutainer containing ethylenediaminetetraacetic acid (EDTA) for other projects. Blood samples were collected following EPA standards and stored in certified PFAS/Teflon-free vials provided by the Wisconsin State Lab of Hygiene (WSLH) (2023 and 2024) or Core for Advanced Water Analysis (CAWA) – UW-Madison (2025). Within 12 h of collection, the blood samples in the heparin tubes were centrifuged at 1200 rpm for 10–12 min to separate plasma from the red blood cells. A glass pipette was used to transfer the plasma to sterile glass tubes. The plasma samples were subject to protein precipitation and then analyzed for PFAS following a PFAS analysis method described in Strom et al. (2025). All samples were stored at -20°C until analysis at the Wisconsin State Laboratory of Hygiene (WSLH) (2023 and 2024) or Core for Advanced Water Analysis (CAWA) (2025).

2.2. Molecular sexing

Bald eagle nestlings were sexed using a previously published protocol for PCR methods (Lee et al., 2010). Briefly, Z and W genes were identified using forward/reverse CHD gene region primers for 94°C for 3min, 30 cycles of [at 94°C for 30sec, 48°C for 30 s, and 72°C for 1min] and 72°C for 10min (Lee et al., 2010). Afterwards, a mixture of 800 μL hiDYE and 100 μL of an Alexa ladder solution was prepared and used as the loading solution. For each sample, 1 μL of PCR product was added to 9 μL of this mixture and fragment analyzed on an ABI 3730 DNA Analyzer. Males have one peak at $\sim 600\text{bp}$ (ZZ), while females have one peak at $\sim 320\text{bp}$ and another at $\sim 600\text{bp}$ (WZ). All samples were run alongside a known male and female bald eagle obtained from a rehabilitation facility in Wisconsin. 46 nestlings were estimated to be female, 61 nestlings were estimated to be male, and 7 nestlings could not be sexed.

2.3. PFAS extraction

Plasma samples were subdivided from their primary stocks and extracted following a modified protein precipitation procedure for liquid tissues (Organtini et al., 2021) while following the WSLH quality control procedures detailed in Strom et al., (2025) and quality control procedures detailed in EPA 1633. 200 μL of each plasma sample was transferred to microcentrifuge tube. An extraction standard mix (Wellington Laboratories, MPFAC-HIF-ES) was diluted 10x and 28.8 μL of the diluted standard was spiked into each sample, followed by 375 μL of HPLC grade acetonitrile. Samples were shaken on a benchtop shaker table at max speed for roughly 5 min, vortexed at max speed for 10 s, then centrifuged at 1400 RCF for 5 min 84 μL of supernatant was transferred to a polypropylene HPLC insert after which 4 μL of 10x diluted non-extraction standard mix (Wellington Laboratories, MPFAC-HIF-IS) was added. Concentrations of all working extraction standard dilutions are listed in S-Table 1. Samples were vortexed for 10 more seconds prior to injection. No solid-phase extraction or further sample cleanup was performed. Where possible, reverse pipetting was used for all transfers. Fetal bovine serum was used for method blanks and precision and recovery checks. When necessary, samples were diluted using standard diluent (4% water, 1% ammonium hydroxide, 0.625% acetic acid, with HPLC grade methanol makeup). For years 2023

and 2024, a nitrogen dry-down and reconstitution step was performed, but after review of the data and impact of the resulting concentration factor, dry-down was deemed not necessary and subsequently not performed in 2025. All materials used in extraction were verified to be appropriately free from target PFAS contamination for each study year. Cleanliness was assessed by examination of extensive process blanks, reagent blanks, and reference matrices.

2.4. Chemical analysis

All samples were analyzed for PFAS either by a contract laboratory (WSLH) or in our research laboratory (CAWA), both in Madison, Wisconsin. Briefly, all measurements were made using high performance liquid chromatography tandem mass spectrometry (LC-MS/MS) using negative mode electrospray ionization. Samples from 2023 were analyzed for 38 PFAS at WSLH, samples from 2024 were analyzed for 39 PFAS at WSLH, and samples from 2025 were analyzed for 40 PFAS at the CAWA (e.g., 45 different PFAS were measured in at least 1 year; 33 PFAS were consistently measured over all three years) (S-Table 2), with the number of target analytes corresponding to changing commercial availability of reference materials. A list of pertinent instrument parameters and ion transitions is included in S-Table 3. A detailed description of the analytical techniques has been described by (Route et al., 2014), and the same general sample processing procedure (Strom et al., 2025) was followed in all study years with exception for the partial evaporation performed in 2023 and 2024. All results were assessed against pertinent QA/QC criteria in EPA 1633a in both WSLH and CAWA (USEPA, 2024), and all criteria for the raw data are within acceptable limits, including extracted standard recovery, non-extracted standard recovery, and routine check standard recovery. Additional quality control procedures included examination of material, process, and method blanks, spiked samples, and repeat injections, all of which within acceptable criteria for WSLH internal procedures (2023, 2024) and within acceptable criteria in EPA 1633a (2025).

Quantitation for all target PFAS is carried out using either extracted internal standard quantitation or isotope dilution, where applicable. Average detection limit for all target PFAS is 0.091 $\mu\text{g/L}$ in 2023 and 2024, and 0.044 $\mu\text{g/L}$ in 2025. While some variability across study years, specifically between 2023 and 2024 and 2025, are apparent in the listed detection limits (S-Table 2), the analysis required to make an assessment of detection limits compliant with EPA 1633 is rigorous and the results demonstrates good agreement in the means and methods across the two study laboratories.

2.5. Data analysis

For all subsequent data analyses, we removed the 12 analytes that were not sampled in all three years for individual analysis. Additionally, we removed 11 analytes for individual analysis due to their low abundance/detection in the samples (e.g., analytes were removed if more than 10% of the birds had levels below the detection limit). Therefore, all remaining analysis was done on 22 PFAS analytes. For samples found to be below the detection limits, we assigned a value of zero in keeping with methods outlined in Strom et al. (2025) and to minimize inflating concentration data. $\sum_{22}$ PFAS was calculated as the total concentration from the 22 analytes. PFAS concentrations were compiled across all

years into a single dataset. Data processing and statistical analyses were conducted in R version 4.3.0 (R Core Team, Vienna, Austria) using the package *dplyr* (Wickham et al., 2025). Regional identifiers (AIS, SSS, FRGB, GBLM, LWR, MWR, UPWR) were recoded as factors to ensure consistent ordering in visualizations. Data are presented as means $\pm$ standard error of the mean (SEM). Significance was set at $p \leq 0.05$.

2.6. Principal components analysis (PCA)

A subset of 22 PFAS analytes, representing precursors, short-chain compounds, and legacy perfluoroalkyl acids and sulfonates, were retained for multivariate analysis, see above. Observations with missing values for any of these analytes were excluded (e.g., birds with missing values were excluded). Principal component analysis (PCA) was performed using the *prcomp* function in R (R Core Team, 2024) with variables standardized to zero mean and unit variance. Contributions of each PFAS to the components were visualized as bar plots (S-Fig. 1).

We then selected the first four principal components, accounting for $\sim 72\%$ of the variance in the PFAS dataset and conducted an unsupervised clustering analysis using the *mclust* package (Scrucca et al., 2023). We used Gaussian finite mixture modeling as implemented in *mclust*, which fits a series of mixture models with varying covariance structures and numbers of clusters (G). The optimal model and number of clusters were determined using the Bayesian Information Criterion (BIC). This likelihood-based method avoids the initialization biases typical of heuristic clustering algorithms (e.g., k-means) and can accommodate unequal cluster size, shape, and orientation.

2.7. PFAS analyte correlation test

Following methods similar to the PCA, a subset of 22 PFAS analytes was retained for analysis and grouped into major chemical classes (precursors, short-chain compounds, legacy acids, legacy sulfonates, and legacy sulfonamides) (S-Table 4) (Buck et al., 2021). For each study region, pairwise Spearman's rank correlations were calculated among all analytes using complete observations. All correlation p-values were adjusted for multiple comparisons using the Benjamini-Hochberg FDR procedure. Correlation coefficients and corresponding adjusted p-values were extracted, and significance levels (significance was set at $p_{adj} \leq 0.05$) were annotated. Regional correlation matrices were assembled to facilitate comparison of co-occurrence patterns across PFAS classes.

2.8. Distance to nearest major water source

Distances were obtained by drawing linear transects from nest locations to the nearest major river system or Great Lake waterbody (Great Lakes, Wisconsin River, Fox River, and Green Bay) using Google Earth Engine (Gorelick et al., 2017), providing a consistent spatial measure across sites. Using *cor.test* in base R (R Core Team, 2024), we applied a custom function with Spearman's rank correlations across multiple PFAS compounds, producing estimates of correlation coefficients, sample sizes, and p-values. Results were summarized by study region and across all of Wisconsin and directionality of Spearman's rho. Significance was set at $p \leq 0.05$.

3. Results

From 2023 to 2025, we collected 115 nestling samples from 65 nesting territories across 7 study locations (Fig. 1A; Table 1). Nestlings ranged from 1 to 3 eaglets in a nest. Nestling age averaged 44.48 days old (range: 26–63 days). The coefficient of variation in age between regions was 17.3%. Nestlings in the UPWR were slightly younger than the other regions (30.12, range: 26.48–35.16); however, the coefficient of variation (CV) was $<12\%$ when removing UPWR. Nestling weight averaged 3.62 kg (range: 2.09–5.53 kg). Nestling age and mass has been shown to have limited impacts on analyte and total PFAS levels (Strom

et al., 2025), thus we do not explore this further here.

From our study regions, the highest levels of PFAS detected in bald eagle nestling blood were in the MWR (412.2 ± 27.1 total PFAS ng/mL; $n = 21$), followed by AIS (330.7 ± 34.5 total PFAS ng/mL; $n = 14$), while the lowest levels were in UPWR (22.9 ± 1.8 total PFAS ng/mL; $n = 5$) (Fig. 1B; S-Table 5). Note, we had low sample sizes from UPWR and thus results from this region should be interpreted with caution. Study regions AIS and SSS consistently showed elevated concentrations of longer chain carboxylate acids such as PFDA, PFNA, PFTrIA, PFUnA. MWR and LWR consistently show elevated concentrations of multiple compounds, including PFOS, PFTeA and PFDoA, and consistently show the presence of precursor compounds such as FOSA, 8:2 FTS, and NetFO-SAA. FRGB and GBLM consistently show elevated levels of PFDA, PFDoA, and PFUnA, although FRGB and GBLM regions did not have the highest concentration of any PFAS analyte as compared to AIS, SSS, MWR, and LWR (Fig. 1B). Within-nest PFAS variation was notable, with some chemicals showing relatively low average coefficients of variation (e.g., PFDA, PFDOA, PFOS, PFUNDA; ~ 0.12 – 0.16) and others showing much higher within-nest variability (e.g., <1.0 for PFPeS and PFBS) (S-Table – 6). Overall, most long-chain legacy acids exhibited less relative variation among siblings within nests, whereas several short-chain and low concentration compounds showed greater relative variability among siblings within nests.

At AIS and SSS, PFOS made up 38.4% and 44.7% of the total PFAS burden respectively. At FRGB, GBLM, LWR, and MWR, PFOS made up 79.1%, 77.1%, 87.4%, and 82.5% of the total PFAS burden, respectively (Fig. 2). At AIS and SSS, PFNA made up 27.0% and 23.8% of the total PFAS burden respectively, while the rest of the regions, PFNA made up less than 5% of total PFAS burden. At AIS and SSS, PFUnA made up 14.1% and 13.0% of the total PFAS burden respectively, while the rest of the regions, PFUnA made up less than 5%. At MWR, LWR, and FRGB, precursor PFAS made up 4.6%, 1.8%, and 1.2% of total PFAS burden, respectively. At AIS and SSS, precursor PFAS made up less than 0.02% of total PFAS burden. Legacy PFAS make up more than 93% of total PFAS burden at all sites (Fig. 2).

PFAS compositional profiles varied across study regions, with separation between legacy sulfonates, precursors, and short-chains (Fig. 3A). Specifically focusing on the differences between AIS and MWR, MWR had the highest levels of 6:2 FTS, 8:2 FTS, NetFOSAA, FOSA, PFNS, PFHPS, PFOS, and PFTeA compared to other regions (Fig. 3A). In contrast, AIS had the highest levels of PFNA, PFUnA, PFDA, PFTrIA, PFPeS, PFHPA, PFBS, and PFOA as compared to the other regions (Fig. 3A).

Unbiased model-based clustering identified five distinct clusters (Fig. 3B) representing unique PFAS contaminant fingerprints – largely across sampling regions. Ordination along components one and two of the PCA provided almost complete separation of AIS from MWR, and partial separation of FRGB (Fig. 3B). PC1 and PC2 combined explained 53.3% of the total variance. PCA3 and onward accounted for less than 12% of the total variance. PC1 showed positive loadings for 13 PFAS compounds, with the strongest being FOSA, NetFOSAA, 8:2FTS, PFNS, and PFOS, and negative loadings for 9 PFAS compounds, with the strongest being PFNA (S-Fig. 1). PC2 showed positive loadings for 18 PFAS compounds, with the strongest being PFTrIA, PFDA, PFUnA, PFNA, PFDoA, PFOA, and negative loadings for 4 PFAS compounds, with the strongest being PFHxA (S-Fig. 1; S-Fig. 2). Using the Gaussian mixture model approach, the optimal number of clusters was determined to be $G = 5$ (best model = VEV, BIC = -1347.12 , ICL = -1356.7 ; Fig. 3b). Mean classification uncertainty was low (0.0835) and mean maximum posterior probability was high (0.965), indicating strong separation among clusters. Cluster sizes ranged from 9 to 43, but were primarily differentiated by magnitude of PFAS burden, driven largely by PFOS, PFBS, and PFUnDA, with Cluster 1 exhibiting markedly elevated concentrations relative to other groups (S-Fig. 2).

The Spearman rank correlation revealed significant associations among quantified PFAS in the plasma of bald eagle nestlings based on

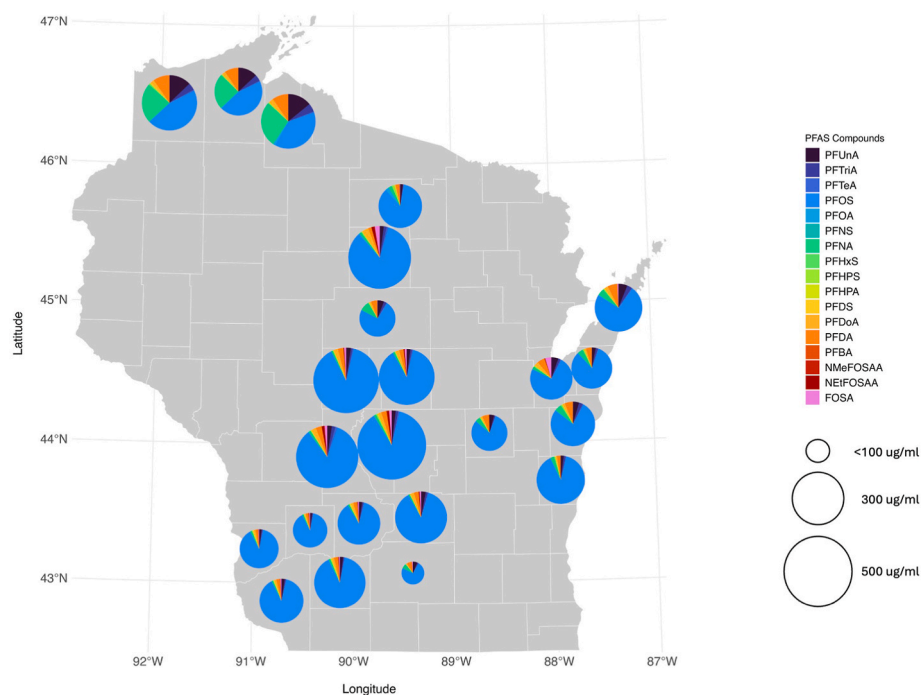


Fig. 2. Regional variation in PFAS concentrations and composition in bald eagle nestlings across Wisconsin. Pie charts show proportional contributions of PFAS compounds; circle size indicates county average total PFAS levels (ng/ml = ppb).

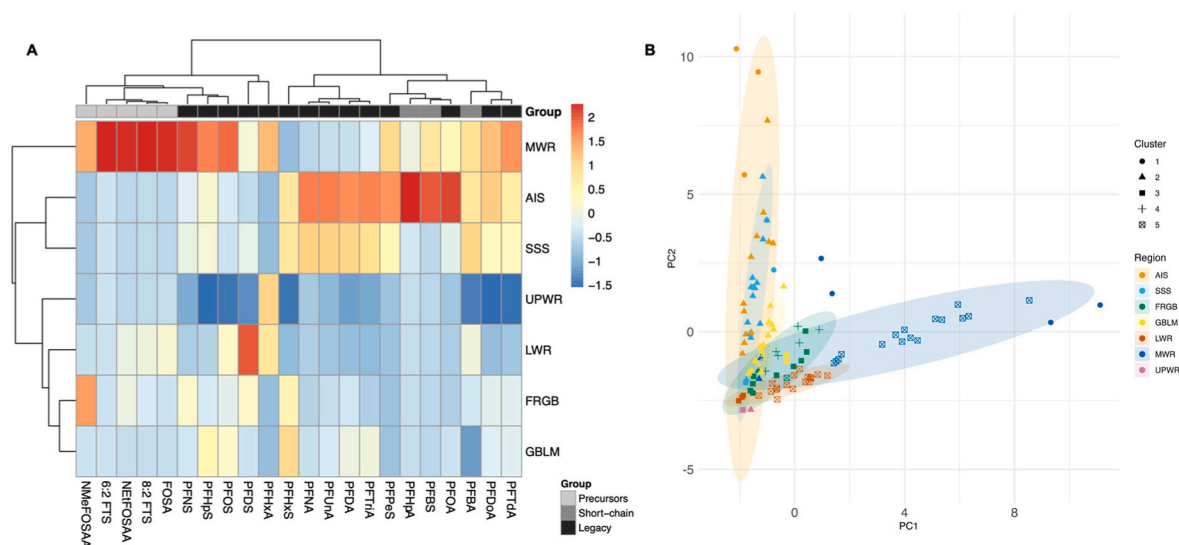


Fig. 3. (A) Heatmap of PFAS composition in bald eagle nestlings across Wisconsin regions. Warmer colors indicate higher relative concentrations (ng/mL = ppb), with clustering by region and PFAS group (precursors, short-chain, legacy) highlighting distinct contamination fingerprints. (B) Principal Component Analysis (PCA) of PFAS Profiles Across Regions. Score plot showing sample clustering by region based on PFAS composition, with distinct groupings highlighted by ellipses. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

region, again particularly emphasizing unique correlations at AIS and SSS compared to MWR (S-Fig. 3; S-Table 7). For AIS, the highest correlations were observed for PFDoA with PFUnA and PFDA ($r_s = 0.99$ and $r_s = 0.97$, respectively) and PFUnA with PFDA and PFNA ($r_s = 0.97$ and $r_s = 0.96$, respectively) all long chain carboxylate acids. For SSS, the highest correlations were observed for PFNA with PFDA and PFUnA ($r_s = 0.97$ and $r_s = 0.98$, respectively) and PFUnA with PFDA ($r_s = 0.99$) again all long chain carboxylic acids. For MWR, the highest correlations were observed for PFTriA with PFTeA ($r_s = 0.97$) two legacy acids and 8:2 FTS with FOSA ($r_s = 0.92$) two precursor compounds. Notably, PFOS showed a significant correlation with PFHPS in all regions except UPWR,

and NEtFOSAA showed a significant correlation with FOSA in MWR, LWR, FRGB, and GBLM.

We observed significant correlations for PFAS and proximity to major waterbodies, however, they differed by region and predominant PFAS components (S-Fig. 4). We observed significant increase PFHxS concentration with increased proximity to the nearest water body in the GBLM region ($p = 0.003$), but observed a negative correlation for PFOS and PFHxS concentrations with increased proximity to the nearest water body for LWR ($p = 0.001$ and $p = 0.03$; respectively) and SSS ($p = 0.005$; for PFHxS). See S-Table 8 for the rest of the patterns for these PFAS in the other regions.

4. Discussion

The concentrations of PFAS are consistently higher in bald eagle plasma than those in other abiotic matrices (e.g., water, sediment) and biotic matrices (e.g., fish tissues, avian eggs) from similar regions (Gkika et al., 2025; Remucal, 2019; Wu et al., 2020). Present results are comparable with past studies that have examined PFAS in eagle plasma, where PFOS levels were near 150–200 ppb (ng/mL = ppb) in the FRGB and GBLM areas (Strom et al., 2025). PFAS bioaccumulates into tissues, and blood commonly shows the highest concentrations in animals (George et al., 2023). Our results highlight bald eagle nestlings as a good species to use as a biomonitoring system for identifying PFAS contamination. Bald eagles are consumer-scavengers with varying diets (e.g., aquatic vs terrestrial) and due to their wide range in food sources, they can elucidate a holistic view of PFAS levels compared to species that are specialists (e.g., finches - granivores), which could miss potential contamination areas. Additionally, because bald eagles have a varying diet - consuming similar foods as people who traditionally harvest off the land - and sit at the top of the food web, they could act as a proxy for understanding the PFAS contamination of humans in this area. Moreover, bald eagle nestlings are fed entirely by their parents within a territory of approximately 5 km diameter, smaller in areas with high food production, which could help identify both regional and fine-scale contamination (Colborn, 1991; Dykstra et al., 2010; Garrett et al., 1993). These results highlight that to understand the health of the entire ecosystem, it is essential to examine both abiotic matrices (e.g., water and soil) and biotic matrices at multiple parts of the food web (e.g., deer, fish, and bald eagles), as each matrix can elucidate parts of the entire contamination puzzle.

Spatial mapping of PFAS concentrations across Wisconsin (Fig. 1A) revealed pronounced regional differences in compound profiles (Fig. 1B; S-Table 5). In all regions, we found that PFOS was the dominant component contributing to the total PFAS burden. Study regions AIS and SSS consistently show elevated concentrations of the same long chain carboxylic acids while study regions MWR and LWR consistently show elevated concentrations of precursor PFAS. These findings suggest that these different regions have unique signatures and could be connected to contamination hotspots, likely reflecting distinct source(s). In AIS and SSS, the predominance of PFDA, PFUnA, and PFNA are consistent with both aqueous film-forming foam (AFFF) legacy inputs (ATSDR, 2021; Dewitt Editor, 2015) and past literature that has shown increased levels of these compounds from atmospheric deposition of fluorotelomer compounds (Remucal, 2019; Xia et al., 2024). In contrast, MWR samples were characterized by elevated levels of both PFOS precursor products (FOSA) and degradation byproducts of PFAS production (e.g., PFHpS), which could be partly consistent with biosolid application in agricultural areas or urban/industrial activity as a diffuse source of contamination (Eriksson et al., 2017; Venkatesan and Halden, 2013). Other regions showed markedly lower PFAS concentrations, though particular PFAS were found in most regions. For example, every region exhibited detectable levels of PFDS, and most regions exhibited detectable levels of PFHpS (except UPWR) and PFHxS (except UWPR). UPWR had the lowest overall PFAS concentrations (albeit sample sizes were low $n = 3$). Low levels of PFAS in the UPWR are consistent with past sampling in UPWR and the Northern Highlands Ecological Landscape ('NHEL') in WI (Strom et al., 2025). Present data further highlights the ability of PFAS to be distributed to remote areas, such as UWPR, from volatilization and degradation of precursors such as fluorotelomer compounds (Remucal, 2019; Xia et al., 2024).

Long-chain carboxylates (PFDA, PFUnA, PFDoDA) and sulfonates (PFOS, PFDS) generally appeared at higher relative concentrations, potentially reflecting their higher bioaccumulation rates and biotransformation potential (Ahrens and Bundschuh, 2014). A short-chain carboxylate, PFBA, was relatively widespread across all regions albeit at low concentrations, consistent with PFBA's increasing role as a replacement for phased-out long-chain PFAS. While PFBA is considered

less bioaccumulative than PFOS, it can be deposited through air and has a higher solubility, leading to an increased potential for long-range transport and ecosystem contamination (Xia et al., 2024). Together, the coexistence of both legacy and replacement compounds in these profiles underscores the ongoing environmental burden from historical production, atmospheric deposition, and current use.

Region-specific PFAS compositions highlight both the similarities within region and the stark differences across regions. Specifically, the PFAS composition patterns in the three counties in northern WI (Fig. 2) further confirm AIS and SSS having a signature of long-chain sulfonates and carboxylates, with PFNA emerging as a notable contributor to the overall contamination in this region. In contrast, the locations associated with the MWR feature PFOS as more than 80% of the total PFAS levels especially in MWR, with precursor compounds such as FOSA, 8:2 FTS, and NetFOSAA at higher proportions compared to other regions. The two counties for FRGB show a more diverse PFAS fingerprint, which includes both precursors and legacy long-chain PFAS. The embedded pie charts (Fig. 2) illustrate these compositional differences at the site/county-level, and these divergences across locations underscore the importance of site-specific sources and land-use practices in shaping PFAS fingerprints. We recognize that county-level comparisons are biologically meaningless; however, were instead intended to average nearby nests which likely have similar exposure patterns.

PFAS compositional profiles can also show subtle differences within proximity of each other. SSS shows similar patterns to AIS, although at lower levels, and SSS does not have relative elevated levels of PFHPA, PFBS, and PFOA. Therefore, AIS may have a slightly different fingerprint compared to SSS despite their proximity. These unique fingerprints could be due different accumulation from food sources (i.e., AIS eaglets' diet potentially entirely aquatic), water movement around the islands, hydrologic structure (e.g., currents, flow, connectivity), or air deposition; though, further research is needed to understand why AIS PFAS composition is slightly different compared to SSS PFAS composition. MWR and LWR were separated from the other regions by higher scores along PC1, while AIS and SSS were separated from the other regions by higher scores along PC2 and FRGB were partially explained by both PC1 and PC2 separating out in the middle. PC1 may be seen as mainly reflecting a point source contamination from the 2018 Husky Superior Refinery Explosion and subsequent fires in Superior, Wisconsin ("CSB issues update on Husky refinery explosion," 2018) or linked to the increased levels of these compounds from atmospheric deposition of fluorotelomer compounds (Remucal, 2019; Xia et al., 2024). PC2 may be seen as reflecting an industrial point source(s) such as wastewater discharge from production or biosolid spreading (Kaeding, 2025). Altogether, such differences in compositional fingerprints indicate that contamination is not uniform across water bodies, even within the same state and can be potentially driven by different contamination source(s) (e.g., as is the case at MWR vs. FRGB vs. AIS) or ecological landscape differences (e.g., as is the case between AIS and SSS) (Ahrens et al., 2015; Loken et al., 2025; Xia et al., 2024).

The Spearman rank correlation revealed significant associations among the quantified PFAS in the plasma of bald eagle nestlings in the different regions. Notably, PFOS showed a significant correlation with PFHPS in all regions except UPWR, and NetFOSAA showed a significant correlation with FOSA in MWR, LWR, FRGB, and GBLM (S-Fig. 3). While PFOS is not believed to biotransform into PFHpS, NetFOSAA can be metabolized by multiple animal species into FOSA (Kolanczyk et al., 2023; Zhang et al., 2025). Therefore, correlations of precursors and other PFAS could be used to identify biotransformation pathways in future research. Overall, understanding how PFAS correlate in specific regions can not only help identify PFAS source fingerprints, but it could potentially provide insight into how PFAS are biotransformed in biota (wildlife). Future research should explore how PFAS fingerprints vary between abiotic and biotic matrices.

Present data suggest the distinctive PFAS fingerprints observed here could be from spatial influences such as proximity to waterbodies (S-

Fig. 4). While proximity to waterbodies did not elucidate clear patterns to PFAS concentrations in all regions, the variability across regions underscores that local factors (e.g., point-source inputs, watershed hydrology, and land-use history) could modulate the strength of these associations (Loken et al., 2025; Peter and Lee, 2025). By using the nearest major river system or Great Lake water body, this metric does not capture localized hotspots associated with smaller water bodies or terrestrial food sources and thus should be interpreted with caution. Together, these findings illustrate how PFAS signatures are shaped by both chemical properties and regional landscape context, reinforcing the importance of incorporating spatial hydrological features into management and monitoring strategies. However, future research should be done to understand how spatial variability influences PFAS contamination.

Environmental monitoring using water, sediment, and fish tissue are common methods for assessing contamination levels in aquatic ecosystems (Remucal, 2019; Scott et al., 2010; Xia et al., 2024). However, these matrices have limitations in that they may not fully capture the extent of exposure to wildlife or humans. Around the US and in the Great Lakes region, many groups have conducted statewide monitoring projects to sample fish tissue and water chemistry at select sites near known or probable sources of PFAS (Stahl et al., 2023). For instance, in 2019, the Wisconsin DNR collected samples from river systems near known or suspected PFAS contamination sites, including Starkweather Creek, the Wisconsin River, the Yahara River, and Lake Superior (<https://dnr.wisconsin.gov/topic/PFAS/SWFish.html>). Despite these comprehensive monitoring efforts, the data collected may not fully represent the exposure levels in wildlife. The presence of contaminants in fish tissue does not entirely correlate with the levels of exposure experienced by wildlife and humans, as factors such as bioaccumulation, dietary habits, and species-specific sensitivities can influence overall exposure levels (Christensen et al., 2016). Therefore, environmental monitoring of water and fish tissues are valuable tools for identifying PFAS signatures and potential contamination hotspots but may not provide a comprehensive picture of the risks to humans and wildlife. A more holistic approach, incorporating biomonitoring and ecological risk assessments of top predators (e.g., bald eagles), is useful to accurately assess and mitigate the impacts of contaminants like PFAS on public health and the environment.

Taken together, these findings highlight the utility of using bald eagle nestlings as a PFAS fingerprinting tool to begin to differentiate contamination source(s) and understand environmental pathways of exposure. PFAS profiles enriched with legacy carboxylates and sulfonates point toward persistent industrial contamination, while those dominated by short-chain acids and precursors reflect the growing role of replacement chemistries and other diffuse inputs (Brunn et al., 2023). From a risk perspective, the persistence of PFOS continues to pose long-term ecological and human health challenges, whereas the widespread occurrence of short-chain acids underscores concerns regarding mobility, long-range transport, and the difficulty of removal (Balgooyen et al., 2026; Christensen et al., 2016; Silver et al., 2023). These results emphasize the need for monitoring strategies that incorporate both legacy PFAS and emerging alternatives, while also accounting for regional differences in industrial history, land use, and proximity to aquatic food sources.

CRedit authorship contribution statement

Gavin Dehnert: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **James Lazarcik:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Sara Haney:** Writing – review & editing, Validation, Methodology, Formal analysis. **Sean M. Strom:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Data curation, Conceptualization. **J. Dylan Maddox:** Writing – review &

editing, Methodology, Data curation. **Felix Grewe:** Writing – review & editing, Resources, Methodology, Funding acquisition. **Emily Cornelius Ruhs:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work the author(s) used ChatGPT in order to refine wording, improve grammar, and assist with code formatting. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2026.124413>.

Data availability

Data are available at the following link: <https://github.com/ecruhs/BAEA-PFAS-fingerprinting.git>

References

- Ackerman Grunfeld, D., Gilbert, D., Hou, J., Jones, A.M., Lee, M.J., Kibbey, T.C.G., O'Carroll, D.M., 2024. Underestimated burden of per- and polyfluoroalkyl substances in global surface waters and groundwaters. *Nat. Geosci.* 17, 340–346. <https://doi.org/10.1038/s41561-024-01402-8>.
- Ahrens, L., Bundschuh, M., 2014. Fate and effects of poly- and perfluoroalkyl substances in the aquatic environment: a review. *Environ. Toxicol. Chem.* 33, 1921–1929. <https://doi.org/10.1002/etc.2663>.
- Ahrens, L., Norström, K., Viktor, T., Cousins, A.P., Josefsson, S., 2015. Stockholm Arlanda Airport as a source of per- and polyfluoroalkyl substances to water, sediment and fish. *Chemosphere* 129, 33–38. <https://doi.org/10.1016/j.chemosphere.2014.03.136>.
- ATSDR, 2021. *Toxicological Profile for Perfluoroalkyls*.
- Balgooyen, S., Mahon, M.B., Krauss, A., Sluka, H., Backe, W.J., Ankley, G.T., Lepak, R.F., 2026. Historical PFAS trends in the Great Lakes using four decades of archived fish. *J. Great Lake. Res.* <https://doi.org/10.1016/j.jglr.2026.102741>.
- Bangma, J., Guille, T.C., Bommarito, P.A., Ng, C., Reiner, J.L., Lindstrom, A.B., Strynar, M.J., 2022. Understanding the dynamics of physiological changes, protein expression, and PFAS in wildlife. *Environ. Int.* <https://doi.org/10.1016/j.envint.2021.107037>.
- Bortolotti, G.R., 1984. Criteria for determining age and sex of nestling bald eagles. *J. Field Ornithol.* 55, 467–481.
- Bowerman, W.W., Roe, A.S., Gilbertson, M.J., Best, D.A., Sikarskie, J.G., Mitchell, R.S., Summer, C.L., 2002. Using bald eagles to indicate the health of the Great Lakes'

- environment. Lakes and reservoirs: science. Policy and Management for Sustainable Use 7, 183–187. <https://doi.org/10.1046/j.1440-1770.2002.00186.x>.
- Brunn, H., Arnold, G., Körner, W., Rippen, G., Steinhäuser, K.G., Valentin, I., 2023. PFAS: forever chemicals—persistent, bioaccumulative and mobile. Reviewing the status and the need for their phase out and remediation of contaminated sites. *Environ. Sci. Eur.* <https://doi.org/10.1186/s12302-023-00721-8>.
- Buck, R.C., Korzeniowski, S.H., Laganis, E., Adamsky, F., 2021. Identification and classification of commercially relevant per- and poly-fluoroalkyl substances (PFAS). *Integrated Environ. Assess. Manag.* 17, 1045–1055. <https://doi.org/10.1002/ieam.4450>.
- Cartron, J.L.E., Gadek, C.R., Dunnun, J.L., Witt, C.C., Campbell, M.L., Romero, S.J., Johnson, A.B., Kutz, J., Wolf, C., Choyke, S.J., Cook, J.A., 2025. Ecosystem-wide PFAS characterization and environmental behavior at a heavily contaminated desert oasis in the southwestern U.S. *Environ. Res.* 279. <https://doi.org/10.1016/j.envres.2025.121872>.
- Christensen, K.Y., Raymond, M., Thompson, B.A., Anderson, H.A., 2016. Perfluoroalkyl substances in older male anglers in Wisconsin. *Environ. Int.* 91, 312–318. <https://doi.org/10.1016/j.envint.2016.03.012>.
- Colborn, T., 1991. Epidemiology of Great Lakes bald eagles. *J. Toxicol. Environ. Health* 33, 395–453. <https://doi.org/10.1080/15287399109531537>.
- Crone, B.C., Speth, T.F., Wahman, D.G., Smith, S.J., Abulikemu, G., Kleiner, E.J., Pressman, J.G., 2019. Occurrence of per- and polyfluoroalkyl substances (PFAS) in source water and their treatment in drinking water. *Crit. Rev. Environ. Sci. Technol.* 49, 2359–2396. <https://doi.org/10.1080/10643389.2019.1614848>.
- CSB issues update on Husky refinery explosion, 2018. C&EN Global Enterprise 96, 19. <https://doi.org/10.1021/cen-09632-polcon5>.
- De Silva, A.O., Armitage, J.M., Bruton, T.A., Dassuncao, C., Heiger-Bernays, W., Hu, X.C., Kärrman, A., Kelly, B., Ng, C., Robuck, A., Sun, M., Webster, T.F., Sunderland, E.M., 2021. PFAS exposure pathways for humans and wildlife: a synthesis of current knowledge and key gaps in understanding. *Environ. Toxicol. Chem.* <https://doi.org/10.1002/etc.4935>.
- Deblonde, T., Cossu-Leguillie, C., Hartemann, P., 2011. Emerging pollutants in wastewater: a review of the literature. *Int. J. Hyg Environ. Health* 214, 442–448. <https://doi.org/10.1016/j.ijheh.2011.08.002>.
- Dewitt Editor, J.C., 2015. *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances, Molecular and Integrative Toxicology*. Springer International Publishing, Cham. <https://doi.org/10.1007/978-3-319-15518-0>.
- Dykstra, C.R., Meyer, M.W., Rasmussen, P.W., Warnke, D.K., 2005. Contaminant concentrations and reproductive rate of Lake Superior bald eagles, 1989–2001. *J. Great Lake. Res.* 31, 227–235. [https://doi.org/10.1016/S0380-1330\(05\)70253-7](https://doi.org/10.1016/S0380-1330(05)70253-7).
- Dykstra, C.R., Route, W.T., Meyer, M.W., Rasmussen, P.W., 2010. Contaminant concentrations in bald eagles nesting on Lake Superior, the upper Mississippi River, and the St. Croix River. *J. Great Lake. Res.* 36, 561–569. <https://doi.org/10.1016/j.jglr.2010.06.006>.
- Dykstra, C.R., Route, W.T., Williams, K.A., 2021. Trends and patterns of perfluoroalkyl substances in blood plasma samples of bald eagle nestlings in Wisconsin and Minnesota, USA. *Environ. Toxicol. Chem.* 40, 754–766. <https://doi.org/10.1002/etc.4864>.
- Eriksson, U., Haglund, P., Kärrman, A., 2017. Contribution of precursor compounds to the release of per- and polyfluoroalkyl substances (PFASs) from waste water treatment plants (WWTPs). *J. Environ. Sci. (China)* 61, 80–90. <https://doi.org/10.1016/j.jes.2017.05.004>.
- Garrett, M.G., Watson, J.W., Anthony, R.G., 1993. Bald eagle home range and habitat use in the Columbia River Estuary, source. *J. Wildl. Manag.*
- George, S.E., Baker, T.R., Baker, B.B., 2023. Nonlethal detection of PFAS bioaccumulation and biomagnification within fishes in an urban- and wastewater-dominant Great Lakes watershed. *Environ. Pollut.* 321. <https://doi.org/10.1016/j.envpol.2023.121123>.
- Gkika, I.S., Arie Vonk, J., ter Laak, T.L., van Gestel, C.A.M., Dijkstra, J., Groffen, T., Bervoets, L., Kraak, M.H.S., 2025. Strong bioaccumulation of a wide variety of PFAS in a contaminated terrestrial and aquatic ecosystem. *Environ. Int.* 202. <https://doi.org/10.1016/j.envint.2025.109629>.
- Gorelick, N., Hancher, M., Dixon, M., Ilyushchenko, S., Thau, D., Moore, R., 2017. Google Earth engine: planetary-scale geospatial analysis for everyone. *Remote Sens. Environ.* 202, 18–27. <https://doi.org/10.1016/j.rse.2017.06.031>.
- Houde, M., Martin, J.W., Letcher, R.J., Solomon, K.R., Muir, D.C.G., 2006. Biological monitoring of polyfluoroalkyl substances: a review. *Environ. Sci. Technol.* 40, 3463–3473. <https://doi.org/10.1021/es052580b>.
- Kaeding, D., 2025. DNR Names Paper Companies Responsible for PFAS Contamination in a Wisconsin County.
- Kelly, B.C., Ikonou, M.G., Blair, J.D., Surridge, B., Hoover, D., Grace, R., Gobas, F.A.P.C., 2009. Perfluoroalkyl contaminants in an arctic marine food web: trophic magnification and wildlife exposure. *Environ. Sci. Technol.* 43, 4037–4043. <https://doi.org/10.1021/es900389a>.
- Koch, A., Kärrman, A., Yeung, L.W.Y., Jonsson, M., Ahrens, L., Wang, T., 2019. Point source characterization of per- and polyfluoroalkyl substances (PFASs) and extractable organofluorine (EOF) in freshwater and aquatic invertebrates. *Environ. Sci. Process. Impacts* 21, 1887–1898. <https://doi.org/10.1039/c9em00281b>.
- Kolanczyk, R.C., Saley, M.R., Serrano, J.A., Daley, S.M., Tapper, M.A., 2023. PFAS biotransformation pathways: a species comparison study. *Toxics* 11. <https://doi.org/10.3390/toxics11010074>.
- Lee, J.C.-I., Tsai, L.-C., Hwa, P.-Y., Chan, C.-L., Huang, A., Chin, S.-C., Wang, L.-C., Lin, J.-T., Linacre, A., Hsieh, H.-M., 2010. A novel strategy for avian species and gender identification using the CHD gene. *Mol. Cell. Probes* 24, 27–31. <https://doi.org/10.1016/j.mcp.2009.08.003>.
- Li, J., Sun, J., Li, P., 2022. Exposure routes, bioaccumulation and toxic effects of per- and polyfluoroalkyl substances (PFASs) on plants: a critical review. *Environ. Int.* 158. <https://doi.org/10.1016/j.envint.2021.106891>.
- Lin, Y., Capozzi, S.L., Lin, L., Rodenburg, L.A., 2021. Source apportionment of perfluoroalkyl substances in Great Lakes fish. *Environ. Pollut.* 290. <https://doi.org/10.1016/j.envpol.2021.118047>.
- Loken, L.C., Corsi, S.R., Alvarez, D.A., Pronschinske, M.A., Lenaker, P.L., Nott, M., Zhang, C., Mani, E., Ankley, G.T., 2025. Per- and polyfluoroalkyl substances in surface water and sediment in Great Lakes tributaries and relations with watershed attributes. *Environ. Toxicol. Chem.* 44, 1503–1524. <https://doi.org/10.1093/etojnl/vga077>.
- Ng, C.A., Hungerbuehler, K., 2015. Exploring the use of molecular docking to identify bioaccumulative perfluorinated alkyl acids (PFAAs). *Environ. Sci. Technol.* 49, 12306–12314. <https://doi.org/10.1021/acs.est.5b03000>.
- Organtini, K.L., Rosnack, K.J., Lame, M.E., Calton, L.J., 2021. *Extracting and Analyzing PFAS from Human Serum*.
- Peter, L.G., Lee, L.S., 2025. Sources and pathways of PFAS occurrence in water sources: relative contribution of land-applied biosolids in an agricultural dominated watershed. *Environ. Sci. Technol.* 59, 1344–1353. <https://doi.org/10.1021/acs.est.4c09490>.
- Podder, A., Sadmani, A.H.M.A., Reinhart, D., Chang, N. Bin, Goel, R., 2021. Per and poly-fluoroalkyl substances (PFAS) as a contaminant of emerging concern in surface water: a transboundary review of their occurrences and toxicity effects. *J. Hazard. Mater.* <https://doi.org/10.1016/j.jhazmat.2021.126361>.
- Point, A.D., Holsen, T.M., Fernando, S., Hopke, P.K., Crimmins, B.S., 2021. Trends (2005–2016) of perfluoroalkyl acids in top predator fish of the Laurentian Great Lakes. *Sci. Total Environ.* 778. <https://doi.org/10.1016/j.scitotenv.2021.146151>.
- R Core Team, 2024. *R: a Language and Environment for Statistical Computing*.
- Remual, C.K., 2019. Spatial and temporal variability of perfluoroalkyl substances in the Laurentian Great Lakes. *Environ. Sci. Process. Impacts* 21, 1816–1834. <https://doi.org/10.1039/c9em00265k>.
- Route, W.T., Key, R.L., Russell, R.E., Lindstrom, A.B., Strynar, M.J., 2014. Spatial and temporal patterns in concentrations of perfluorinated compounds in bald eagle nestlings in the upper Midwestern United States. *Environ. Sci. Technol.* 48, 6653–6660. <https://doi.org/10.1021/es501055d>.
- Scott, B.F., de Silva, A.O., Spencer, C., Lopez, E., Backus, S.M., Muir, D.C.G., 2010. Perfluoroalkyl acids in Lake Superior water: trends and sources. *J. Great Lake. Res.* 36, 277–284. <https://doi.org/10.1016/j.jglr.2010.03.003>.
- Scrucca, L., Fraley, C., Murphy, T., Raftery, A., 2023. *Model-Based Clustering, Classification, and Density Estimation Using Mclust in R*.
- Silver, M., Phelps, W., Masarik, K., Burke, K., Zhang, C., Schwartz, A., Wang, M., Nitka, A.L., Schutz, J., Trainor, T., Washington, J.W., Rheineck, B.D., 2023. Prevalence and source tracing of PFAS in shallow groundwater used for drinking water in Wisconsin, USA. *Environ. Sci. Technol.* 57, 17415–17426. <https://doi.org/10.1021/acs.est.3c02826>.
- Stahl, L.L., Snyder, B.D., McCarty, H.B., Kincaid, T.M., Olsen, A.R., Cohen, T.R., Healey, J.C., 2023. Contaminants in fish from U.S. rivers: probability-based national assessments. *Sci. Total Environ.* 861. <https://doi.org/10.1016/j.scitotenv.2022.160557>.
- Strom, S.M., Wojcik, B., Dehnert, G., Cornelius Ruhs, E., 2025. Longitudinal trends of per- and poly-fluoroalkyl substances (PFAS) in nestling bald eagles (*Haliaeetus leucocephalus*) from Wisconsin. *Environ. Res.* 276, 121468. <https://doi.org/10.1016/j.envres.2025.121468>.
- USEPA, 2024. Method 1633, Revision A Analysis of per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS.
- Venkatesan, A.K., Halden, R.U., 2013. National inventory of perfluoroalkyl substances in archived U.S. biosolids from the 2001 EPA National Sewage Sludge Survey. *J. Hazard. Mater.* 252–253, 413–418. <https://doi.org/10.1016/j.jhazmat.2013.03.016>.
- Watson, J.W., 2002. *Comparative Home Ranges and Food Habits of Bald Eagles Nesting in Four Aquatic Habitats in Western Washington*. Source. Winter.
- Wickham, H., François, R., Henry, L., Müller, K., Vaughan, D., 2025. *Dplyr: a Grammar of Data Manipulation*.
- Witt, C.C., Gadek, C.R., Cartron, J.L.E., Andersen, M.J., Campbell, M.L., Castro-Farías, M., Gyllenhaal, E.F., Johnson, A.B., Malaney, J.L., Montoya, K.N., Patterson, A., Vinciguerra, N.T., Williamson, J.L., Cook, J.A., Dunnun, J.L., 2024. Extraordinary levels of per- and polyfluoroalkyl substances (PFAS) in vertebrate animals at a New Mexico desert oasis: multiple pathways for wildlife and human exposure. *Environ. Res.* 249. <https://doi.org/10.1016/j.envres.2024.118229>.
- Wu, Y., Simon, K.L., Best, D.A., Bowerman, W., Venier, M., 2020. Novel and legacy per- and polyfluoroalkyl substances in bald eagle eggs from the Great Lakes region. *Environ. Pollut.* 260, 113811. <https://doi.org/10.1016/j.envpol.2019.113811>.
- Xia, C., Capozzi, S.L., Romanak, K.A., Lehman, D.C., Dove, A., Richardson, V., Greenberg, T., McGoldrick, D., Venier, M., 2024. The ins and outs of Per- and polyfluoroalkyl substances in the Great Lakes: the role of atmospheric deposition. *Environ. Sci. Technol.* 58, 9303–9313. <https://doi.org/10.1021/acs.est.3c10098>.
- Zhang, J., Cioni, L., Jaspers, V.L.B., Asimakopoulos, A.G., Peng, H.B., Ross, T.A., Klaassen, M., Herzke, D., 2025. Shellfish and shorebirds from the East-Asian Australian flyway as bioindicators for unknown per- and polyfluoroalkyl substances using the total oxidizable precursor assay. *J. Hazard. Mater.* 487. <https://doi.org/10.1016/j.jhazmat.2025.137189>.