



PFAS exposure linked to altered innate immune biomarkers in a sentinel apex predator, the bald eagle (*Haliaeetus leucocephalus*)

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ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are persistent and ubiquitous environmental contaminants that can bioaccumulate in food webs, yet their effects on wildlife health—particularly in apex predators—remain poorly characterized. Bald eagles (*Haliaeetus leucocephalus*), which integrate contaminant exposure across aquatic and terrestrial prey, provide a valuable sentinel for assessing biological responses to PFAS in freshwater ecosystems. Here, we evaluated associations between PFAS levels in blood plasma of bald eagle nestlings sampled over multiple years across Wisconsin, USA, and biomarkers of innate immune function, including naturally circulating antibodies (IgY), complement lysis ability, and total white blood cell counts (WBC). To account for potential non-linear exposure–response relationships, we modeled associations between PFAS concentrations and immune biomarkers using a suite of candidate functional forms. Results indicate that a subset of individual PFAS exhibited significant associations with immune biomarkers after covariate adjustment, with predominantly negative relationships observed between several long-chain PFAS and complement-mediated lysis, alongside fewer but detectable associations with IgY. In contrast, complement activity was more frequently associated with PFAS exposure than humoral immunity, and exposure–response shapes varied across analytes, with log-linear and other non-linear forms often providing the best fit. In analyses of leukocyte profiles, heterophil proportions were positively associated with multiple PFAS structural groupings—including long-chain, short-chain, sulfonate, carboxylate, and legacy PFAS—while one individual analyte (PFHxA) showed a negative association, suggesting selective shifts in innate immune cell composition rather than broad changes in total leukocyte counts. Collectively, these findings suggest that environmentally relevant PFAS concentrations are associated with altered immune biomarkers in a sentinel apex predator, highlighting potential implications for wildlife health in contaminated freshwater ecosystems.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a diverse class of highly persistent synthetic chemicals that are widely used in industrial applications and consumer products (De Silva et al., 2021). Due to their chemical stability and resistance to degradation, PFAS are globally distributed in soils, surface waters, groundwater, and biota, raising concerns about their potential biological effects at environmentally relevant concentrations (Crone et al., 2019; Koch et al., 2019; Podder

et al., 2021). Environmental releases occur from both point sources (e. g., manufacturing facilities, wastewater treatment plants, landfills, fire-fighting training sites) and diffuse pathways (e.g., atmospheric deposition), resulting in contamination across terrestrial and freshwater ecosystems. Although regulatory efforts have historically emphasized individual compounds such as PFOS and PFOA through drinking water, surface water, and soil standards (US EPA, 2021; WI DNR, 2022), wildlife are exposed to complex PFAS mixtures that vary in chain length and functional chemistry (Dehnert et al., 2026). Understanding the

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biological consequences of these environmentally relevant mixtures and concentrations remains a major research gap.

In the Great Lakes region, PFAS detections have increased substantially over the past decade, including across Wisconsin (Dehnert et al., 2026; Dykstra et al., 2021; Strom et al., 2025). Because PFAS can bioaccumulate and biomagnify, species higher up the trophic level may experience elevated exposure relative to their surrounding environments. The bald eagle (*Haliaeetus leucocephalus*) is an apex predator of aquatic and nearshore food webs and because they are scavengers that consume diverse prey sources (e.g., aquatic and terrestrial prey), they make an effective sentinel species for terrestrial and freshwater ecosystem contamination (Bowerman et al., 2002; Dehnert et al., 2026; Dykstra et al., 2021; Route et al., 2014; Strom et al., 2025). Long-term monitoring programs have demonstrated that bald eagles can be sensitive to environmental contaminants and can reveal spatial patterns of exposure and sublethal biological effects (Bowerman et al., 2002; Carson, 1962; Dehnert et al., 2026).

While reproductive impairment from legacy contaminants in birds is well documented (e.g., DDT, DDE), comparatively little is known about the physiological consequences of PFAS exposure in free-ranging wildlife, particularly apex predators (Custer et al., 2019, 2014, 2013; Robuck et al., 2020). Most avian PFAS studies have focused on occurrence patterns and, to a lesser extent, reproductive endpoints (Custer et al., 2019, 2012, 2013), with limited investigation into health-related biomarkers (Karouna-Renier et al., 2026). However, there is accumulating evidence from laboratory rodent studies and human studies showing that PFAS can modulate immune function, including altered antibody production, disrupted inflammatory gene signaling, dysregulation of immune homeostasis, and changes in immune gene expression (Castaño-Ortiz et al., 2019; DeLeon et al., 2020; Fernie et al., 2005; Guillette et al., 2022a; von Holst et al., 2021). To date, only one study has reported immunomodulatory effects of PFAS in a free-ranging avian species, documenting associations between PFOS exposure and altered plasma protein levels and erythrocytic abnormalities in little penguins (*Eudyptula minor*) (Wells et al., 2024), but see (Karouna-Renier et al., 2026). Altogether, this underscores the limited understanding of PFAS-immune relationships under natural exposure conditions in wildlife.

The immune system plays a critical role in maintaining organismal health and mediating resistance to pathogens and parasites, particularly during early life stages when individuals may be especially vulnerable (Lochmiller and Deerenberg, 2000; Sheldon and Verhulst, 1996). Sublethal alterations in immune biomarkers can precede measurable impacts on survival or reproduction and therefore can serve as early indicators of contaminant-induced physiological stress. In regions such as the Great Lakes, where wildlife are concurrently exposed to chemical contaminants and emerging infectious diseases—including pathogenic avian influenza (low-PAI and high-PAI)—understanding contaminant-related immune modulation is particularly urgent (Acevedo-Whitehouse and Duffus, 2009; Chou et al., 2025; Ross et al., 2025).

Herein, we investigated associations between PFAS exposure and immune biomarkers in bald eagle nestlings sampled across Wisconsin, USA, over a three-year period (2023–2025). We assessed baseline immune function using measures of naturally circulating antibodies (IgY), complement lysis, total white blood cell counts, and individual cell-type proportions compared to quantified concentrations of multiple PFAS analytes in plasma. By evaluating relationships across individual PFAS analytes and functional groupings, and by allowing for non-linear exposure–response relationships, this study aims to characterize how environmentally relevant PFAS mixtures correspond with indicators of health and physiological function in a sentinel apex predator. These findings contribute to a growing body of evidence linking environmental PFAS exposure to biological responses in wildlife and can provide context for assessing potential risks within freshwater ecosystems.

2. Materials and methods

2.1. Sampling

Fieldwork was conducted from 2023 to 2025 throughout the state of Wisconsin, USA during the bald eagle breeding season (May/June), under the USGS federal banding permit 05322 and University of Wisconsin – Madison IACUC protocol L006878-A01. In 2023, nests were sampled from the Green Bay, Lake Superior South Shore, and Apostle Island areas (Fig. 1). In 2024, nests were sampled from the Green Bay, Door County, Superior South Shore, and Apostle Island areas. In 2025, nests were sampled along the Wisconsin River (Fig. 1).

To retrieve the nestlings, a trained arborist climbed up to the nest, removed the nestlings from the nest one at a time, and lowered them down to a field team on the ground. Nestlings were weighed, biometric measurements were taken, 3–4 breast feathers were sampled, and a blood sample was collected before being returned to the nest. 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, 2004; Bowerman et al., 2002; Dykstra et al., 2005). The age of the nestlings was estimated by the length of their 8th primary feather (Bortolotti, 2004).

A blood sample was taken from each nestling using a 21-gauge butterfly needle in the brachial vein. Less than 1% of the bird's total body weight in blood was taken and stored in both purple (EDTA additive) and green (Heparin additive) top collection tubes for later analysis. Nestlings were banded with a USGS aluminum band and returned to their nests. 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. All samples were stored at -20°C until they were analyzed for PFAS.

2.2. Sample contaminant analysis

All samples were analyzed for PFAS either by the Wisconsin State Laboratory of Hygiene or the Core Facility for Advanced Water Analysis, both in Madison, Wisconsin. Briefly, all measurements were performed using high performance liquid chromatography tandem mass spectrometry with quantification using turbo ion spray triple quadrupole mass spectrometer in the negative ionization mode. 38–40 PFAS analytes were analyzed (Table S1), depending on the study year (i.e., 45 different PFAS were measured in at least 1 year; 33 PFAS were consistently measured over all three years) using a modified EPA1633a method (USEPA, 2024). The expanded methods can be found in Dehnert et al. (2026). All results were assessed against pertinent QA/QC criteria in EPA 1633a (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. 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.

2.3. Molecular sexing

Bald eagle nestlings were sexed using two previously published protocols (Dehnert et al., 2026; Lee et al., 2010). Briefly, Z and W genes were identified using forward/reverse CHD gene region primers. Afterward, fragment analysis was performed on a 3730xl sequencer using a custom size standard (Maddox and Feldheim, 2014). Males were identified by the presence of a single band at $\sim 600\text{bp}$ (ZZ), whereas females exhibited two bands: one at $\sim 320\text{bp}$ (W) and one at $\sim 600\text{bp}$ (Z);

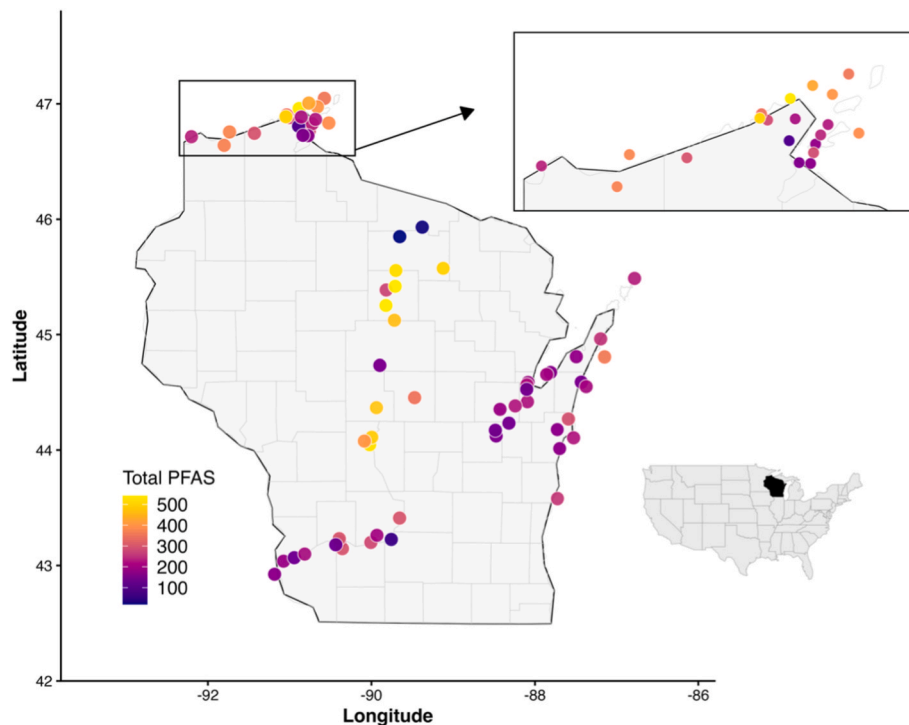


Fig. 1. Spatial distribution of nest-level total PFAS concentrations across Wisconsin eagle nests (2023-2025). Points represent nest-year locations (slightly jittered to reduce overlap) and colored by mean total PFAS concentration (ng/mL).

exemplified in Fig. S1). All samples were run alongside a known male and female bald eagle obtained from a rehabilitation facility in Wisconsin.

2.4. Molecular parasite detection

During WBC counts, the number of red blood cells infected with blood parasites was counted, if present. Four common blood parasites were screened: *Haemoproteus*, *Plasmodium*, *Leucocytozoon*, and *Trypanosoma* using polymerase chain reaction (PCR; Supplemental Table 2). The number of cells infected is reported per 10,000 RBCs. We also screened each bird for blood parasites using three primer sets using nested PCR. For PCR1, we based our protocol on Waldenström et al. (2004) using first-round primers HAEMNF and HAEMNR2 and second-round primers HAEMF and HAEMR2. For PCR2, we based our protocol on Hellgren et al. (2004) using first-round primers HAEMNF1 and HAEMNR3, and second-round primers HAEMFL and HAEMR2L. For PCR3, we based our protocol on Sehgal et al. (2006) using first-round primers DW2 and DW4, and second-round primers LeucoF and LeucoR. This combination of primer sets was implemented to reduce the high rate of false-negative detections typically known to occur using only one nested protocol (per communication with H. Skeen, Field Museum, Chicago, IL). The same PCR conditions were used as in the molecular sexing protocol; however, during the second round of each protocol, 4.0 µl of PCR1 product was used as the template for PCR2. Primer sequences and thermocycler profiles are provided in Table S1.

2.5. Health parameters

2.5.1. White blood cell differentials

The number of circulating leukocytes can be used as a general measure of health and innate immunity. Blood smears were made in the field, immediately air-dried, fixed with 100% methanol, and stained with Wright-Giemsa stain (Owen, 2011). Smears were viewed under 100x magnification with oil immersion to complete the leukocyte profile (Campbell and Dein, 1984). Lymphocytes, heterophils, eosinophils,

monocytes, and basophils were counted, with a total of 50 fields of view at 100% coverage (Owen, 2011). The total white blood cell (WBC) counts were then calculated out of 10,000 red blood cells. Due to a staining issue, there are no reportable data for WBC counts from the eagles sampled in 2024, thus sample size for WBC analysis is $n = 70$ birds ($n = 25$ from 2023 and $n = 45$ from 2025).

2.5.2. Circulating IgY antibodies

Serum samples were used to assess circulating baseline antibody (IgY) levels using an enzyme-linked immunosorbent assay (ELISA) (Cornelius et al., 2017). In short, we coated a 96-well plate with 1 µl serum diluted in 99 µl coating buffer (in duplicate) and incubated the plate overnight (~16 h) at 37°C. After the incubation, coating solution was removed, and 200 µl of block buffer was added to each well and incubated at room temperature for 30 min. The plate was then washed four times with ELISA wash buffer. Then we added 50 µl of goat anti-bird IgG (Bethyl A140-110P) diluted in 1:1000 blocking buffer. The plate was again washed four times. 100 µl of ABTS substrate was added to each well and incubated at room temperature, in the dark for 5 min 100 µl of 1% SDS was added across the plate and read with an Epoch spectrophotometer at $\lambda = 405$ nm.

2.5.3. Complement lysis

To measure complement lysis ability, we used an in vitro assay that employs spectrophotometry to detect complement-lysis activity (Cornelius et al., 2017). This assay is used to assess the eagle's ability to neutralize (lyse) a foreign "pathogen" (Matson et al., 2005; Merchant et al., 2006). We followed methods previously outlined (Cornelius et al., 2017; Killpack et al., 2015). Briefly, 20 µl of plasma was diluted into 60 µl of PBS (in duplicate). Then 80 µl of a 2% rabbit red blood cell (RRBC) solution was added to each sample dilution. Positive control samples were created by adding 20 µl heat-inactivated plasma diluted in 60 µl PBS to 80 µl of a 1% Triton-X-100/2% RRBC solution. Negative control samples consisted of a 1:1 PBS and rabbit red blood cell solution. Plasma blanks were created by adding 20 µl heat-inactivated plasma diluted in 60 µl PBS to 80 µl of the 2% rabbit RBCs. All samples (and

controls) were then incubated in a water bath set at 56°C for 30 min. After incubation, samples were centrifuged for 2 min at 2000xg to form a pellet of un-lysed RBCs. Forty microliters from each tube were then loaded (in triplicate) onto a 96-well plate. The plate was then read at 540 nm, and optical density (OD) values are reported as (OD of sample)-(OD of negative control) for percent lysed. While rabbit red blood cells are a widely used standard substrate for this assay in avian ecology, cross-species reactivity of complement proteins is not perfect, and this assay may not fully capture the functional complement activity relevant to pathogen defense in eagles specifically.

2.6. Statistical analyses

2.6.1. Data processing and variable construction

All analyses were conducted in R version 4.5.2 (R Core Team, 2024). PFAS exposure variables were considered both individually (analyte-level) and as grouped metrics, including functional class (e.g., carboxylates, sulfonates), carbon chain length (short vs. long), and legacy versus emerging compounds (Buck et al., 2021). Summed concentrations were calculated as the total of constituent analytes within each category. Values below detection limits were treated as zero to represent non-detects.

Immune response variables (IgY and complement lysis) were log-transformed to improve normality and stabilize variance. PFAS predictors were standardized prior to modeling. A small constant (1×10^{-6}) was added to PFAS variables where necessary to allow log transformation and fitting of power functions.

2.6.2. Model structure and functional form testing

We used a two-stage modeling approach to evaluate associations between PFAS exposure and immune responses. Candidate functional forms were chosen to reflect the breadth of biologically plausible exposure-response relationships in toxicological systems, where dose-response curves frequently exhibit non-linearity, threshold effects, or saturable dynamics that linear models fail to capture (Lagarde et al., 2015; Ritz, 2010). In the first stage, we identified the best-supported functional form for each PFAS-immune pairing from five candidates: linear, log-linear, quadratic, exponential, and power functions. PFAS predictors with fewer than eight observations during exploratory shape analyses were excluded from consideration. Models returning fitting errors were excluded from model comparison. Model fit was evaluated using AIC, and the lowest-AIC model was selected. This form-selection step was motivated by biological inference rather than exploratory fitting — each candidate form corresponds to a distinct toxicological mechanism (e.g., log-linear for receptor saturation, quadratic for non-monotonic responses).

2.6.3. Mixed-effects modeling of PFAS-immune associations

In the second stage, the selected functional form for each PFAS variable was incorporated into linear mixed-effects models to evaluate associations with immune responses while accounting for biological covariates and hierarchical structure. All models included fixed effects for PFAS exposure, sex, and age, and a random intercept for nest identity. PFAS predictors were standardized to z-scores prior to mixed-effects modeling to facilitate comparison of effect sizes among analytes. Models were fit using maximum likelihood estimation. Observations with missing outcome variables, PFAS concentrations, or covariates were excluded on a model-by-model basis. Models returning fitting errors were excluded from inference. Statistical significance of PFAS effects was assessed using model-derived p-values. To account for multiple comparisons across PFAS variables, false discovery rate (FDR) correction was applied simultaneously across all PFAS-immune pairings within each immune endpoint, including non-significant results, ensuring that the correction reflects the full multiple comparisons burden rather than being applied selectively to significant findings. To assess sensitivity to functional form selection, all mixed-effects models

were refit using log-linear specifications for every PFAS predictor regardless of AIC-selected form (Supplemental Table 3). Results were highly consistent with the primary analysis: 3 of 4 IgY associations and 9 of 10 FDR-significant complement lysis associations remained significant, with concordant effect directions, supporting the robustness of findings to modeling choices (Supplemental Tables 4 and 5, respectively).

2.6.4. Interaction analyses

To evaluate whether PFAS-immune relationships differed by host characteristics, we extended the mixed-effects models to include interaction terms between PFAS exposure and sex and age. Functional forms for PFAS variables were retained from the first-stage model selection. Models included fixed effects for PFAS, sex, age, and their interactions, along with a random intercept for nest identity. Significance of interaction terms was assessed using model p-values with FDR correction applied within each set of tests.

2.6.5. Associations between PFAS exposure and leukocyte metrics

Associations between PFAS exposure and leukocyte profiles were evaluated using generalized linear mixed-effects models in R (glmmTMB, broom.mixed) with nest identity included as a random intercept and age and sex as fixed effects (Bolker et al., 2026; Brooks et al., 2026). PFAS predictors included individual analytes and total PFAS concentrations.

Leukocyte proportions (lymphocytes, heterophils, eosinophils, basophils, and monocytes) were modeled using beta distributions with a logit link after transforming values to the open interval (0,1). Total white blood cell counts were modeled using a negative binomial distribution with a log link. For each PFAS-outcome pairing, candidate PFAS functional forms (linear, log-transformed, quadratic) were compared using AIC, and the best-fitting form was retained. Models that failed to fit successfully were excluded from model comparison.

Model significance was evaluated using likelihood ratio tests comparing full models (including PFAS and covariates) to reduced models including covariates only. P-values were adjusted for multiple testing using the Benjamini-Hochberg false discovery rate correction within each outcome class.

3. Results

3.1. Overview

From 2023 to 2025, 69 nests were sampled with an average of 1.87 nestlings per nest (range = 1-3 nestlings). Of the 116 birds sampled, molecular sexing based on the CHD gene and fragment analysis identified 52 females, 61 males, and 3 unknown sex birds. The average age of nestlings was 45.15 days (range: 26.48-63.3 days). Birds from Adams County (Middle Wisconsin River, $n = 3$) tended to be the youngest with an average age of 29.93 days, while birds from Douglas and Columbia counties tended to be the oldest (59.61 and 59.1 days, $n = 7$ and $n = 2$, respectively). Door county (near Lake Michigan) had the greatest range of nestling age with an average of 43.6 days ($n = 11$, stdev = 10.3 days).

3.2. Molecular parasite detection

We did not detect any blood parasites in any of the samples.

3.3. Concentration of PFAS analytes across Wisconsin

Levels of contamination were previously reported in Dehnert et al. (2026). Briefly, across 116 bald eagle nestlings sampled from seven regions of Wisconsin and the Great Lakes (2023–2025), PFAS were detected in all birds, with total concentrations varying widely by region and reaching the highest levels in the Middle Wisconsin River and Apostle Islands (Fig. 1; Table 1). Regional PFAS profiles differed

Table 1

Summary values from the top 19 PFAS analytes.

Abbreviation	Full_Name	Mean_ng_mL	Min_ng_mL	Max_ng_mL
FTSA8:2	1H,1H,2H,2H-Perfluorohexanesulphonic acid	2.027	0	31.616
FOSA	Perfluorooctanesulfonamide	1.5729	0	13.388
NeTFOSAA	N-ethylperfluorooctane sulfonamidoacetic acid	1.1092	0	15.505
NMeFOSAA	N-methylperfluorooctane sulfonamidoacetic acid	0.1034	0	0.818
PFBA	Perfluorobutanoic acid	0.0926	0	0.853
PFDA	Perfluorodecanoic acid	14.898	1.773	61
PFDoA	Perfluorododecanoic acid	5.865	0.597	19.1
PFDS	Perfluorodecane sulfonic acid	1.0606	0.12	4.217
PFHpA	Perfluoroheptanoic acid	0.0377	0	1.56
PFHpS	Perfluoroheptanesulfonic acid	1.3028	0	9.992
PFHxA	Perfluorohexanoic acid	0.0081	0	0.043
PFHxS	Perfluorohexanesulfonic acid	0.925	0.104	6.37
PFNA	Perfluorononanoic acid	25.2603	0.731	149
PFNS	Perfluorononanesulfonic acid	0.1447	0	0.757
PFOA	Perfluorooctanesulfonic acid	1.2136	0	29.3
PFOS	Perfluorooctane sulfonic acid	186.73	11.988	502.62
PFTeDA	Perfluorotetradecanoic acid	1.5993	0	5.803
PFTrDA	Perfluorotridecanoic acid	6.8488	0.549	34.1
PFUnDA	Perfluoroundecanoic acid	17.3349	2.063	97.3

markedly in composition, with most sites dominated by PFOS, some sites having elevated precursor compounds (e.g., 8:2 FTS and NeTFOSAA), and some sites showing higher proportions of long-chain carboxylate PFAS (e.g., PFNA and PFUnA). Multivariate analyses (PCA and mixture modeling) identified five distinct contaminant fingerprints that largely corresponded to geographic regions, indicating spatially structured PFAS exposure patterns.

3.4. Mixed-effects modeling of PFAS-immune associations

Across both IgY and complement lysis, PFAS-immune associations were heterogeneous in functional model form and significance (Supplemental Tables 4 and 5, respectively). For IgY, quadratic and log-linear relationships were most common (Fig. 2 and S2), though several PFAS (e.g., PFHxA, PFNA, next-gen, PFOS) were best fit by power or exponential models and were highly significant, with LRT p-values often <0.001. Most relationships were negative, though occasionally these relationships showed weaker positive correlations with PFAS (e.g., PFDA, PFNA, PFHxS). Individual compounds like PFBA and PFDA also showed negative log-linear associations. Category-level sums sometimes exhibited negative non-linear relationships and were occasionally significant (e.g., amine, sulfonic, and next-gen). Total PFAS load had a negative quadratic association with IgY.

For complement lysis, log-linear models were frequently the best fit for individual PFAS (e.g., NeTFOSAA, PFDA, PFNA, PFHxS), while some PFAS (PFBA, NMeFOSAA, next-gen) followed power or exponential patterns (Fig. 3 and S3). Quadratic models explained a few associations (PFDS, PFOS, short-chain), with significance varying across analytes. Total PFAS load showed a nonsignificant quadratic relationship with complement lysis.

Overall, non-linear functional forms (power, exponential, quadratic) were important for capturing exposure-response patterns in certain PFAS, while log-linear trends predominated for many PFAS. This highlights that PFAS effects on immune markers are both analyte- and marker-specific, with some compounds driving strong non-linear responses.

3.5. Statistical analysis of PFAS and immune markers

3.5.1. IgY

After controlling for sex, age, and nest identity, relatively few PFAS showed statistically supported associations with IgY concentrations, and detected effects were primarily linear or log-transformed (Supplemental Table 6; Fig. S4). PFBA was negatively associated with IgY under a log-transformed model (estimate = -0.12, SE = 0.039, p = 0.0039, FDR p =

0.023), indicating lower IgY concentrations with increasing exposure. Strong associations were observed for PFHxA and PFHxS, both best described by log models: PFHxA exhibited a significant negative relationship with IgY (estimate = -0.21, SE = 0.040, p < 0.001, FDR p = 0.00021), whereas PFHxS showed a positive association (estimate = 0.18, SE = 0.050, p = 0.00063, FDR p = 0.007). PFNA and PFOA showed modest positive trends (PFNA: estimate = 0.095, SE = 0.055, p = 0.086; PFOA: estimate = 0.096, SE = 0.046, p = 0.043), though neither remained significant after the FDR correction. Quadratic models suggested non-linear relationships for PFBS, PFNS, and total PFAS, indicating a potential U-shaped or saturating effect. FDR-significant IgY associations were modest in magnitude, corresponding to 10–20% changes in antibody levels per standard deviation increase in PFAS concentration. Chain length showed weak trends: longer chain PFAS were negatively associated with IgY (estimate = -0.077, SE = 0.041, p = 0.060), while next-generation PFAS exhibited a stronger negative association (estimate = -0.13, SE = 0.038, p = 0.0016, FDR p = 0.013). All remaining PFAS—including PFDA, PFDOA, PFDS, PFHpA, PFHpS, PFPES, PFTRdA, PFUNdA, PFTeDA, and short-chain or functional subtypes—showed no statistically significant associations with IgY (FDR p > 0.05). Overall, these results indicate that only a small subset of PFAS compounds were associated with variation in humoral immunity, with both positive and negative exposure-response relationships observed and limited evidence supporting non-linear effects in the final adjusted models.

3.5.2. Complement lysis

After controlling for sex, age, and nest identity, several PFAS exhibited significant associations with complement-mediated lysis, although most compounds showed no detectable relationship after multiple-comparison correction (Supplemental Table 7; Fig. S4). PFHxA demonstrated the strongest positive association with complement lysis (estimate = 1.29 ± 0.26, p < 0.001, FDR p = 0.00021), indicating higher lysis capacity with increasing exposure. In contrast, multiple PFAS were negatively associated with complement lysis following log-transformed models, including PFDA (estimate = -1.20 ± 0.28, p < 0.001, FDR p = 0.0008), PFHxS (-1.02 ± 0.29, p = 0.0010, FDR p = 0.0036), PFNA (-1.06 ± 0.30, p = 0.00079, FDR p = 0.0036), PFNS (-0.78 ± 0.27, p = 0.0057, FDR p = 0.014), PFOA (-0.93 ± 0.26, p = 0.0011, FDR p = 0.0036), PFTRdA (-1.00 ± 0.27, p = 0.00057, FDR p = 0.003), and PFUNdA (-1.12 ± 0.27, p < 0.001, FDR p = 0.001). Among FDR-significant associations, predicted effect sizes for complement lysis were substantial: most negatively-associated analytes (PFDA, PFHxS, PFNA, PFOA, PFTRdA, PFUNdA) corresponded to 55–70% decreases in complement activity per one standard deviation increase in

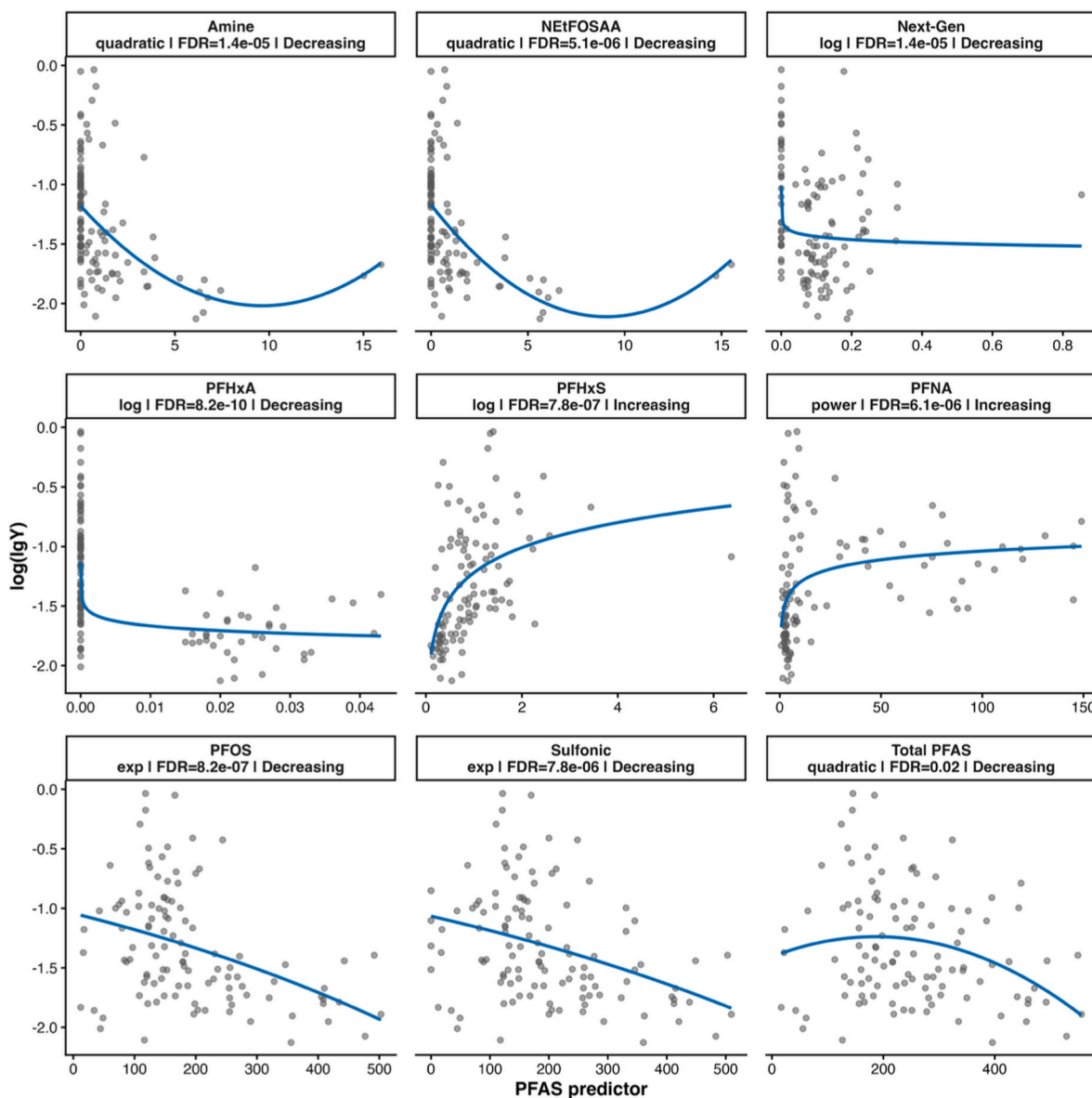


Fig. 2. Key PFAS predictors of humoral immunity (IgY) in eagles. Panel show the nine selected PFAS-IgY relationships, for the most significant functional groupings and the top-ranked associations by false discovery rate (FDR). Lines represent the best-fit models (linear, log, quadratic, exponential, or power).

PFAS concentration. A negative quadratic relationship was observed for short-chain PFAS, which remained significant after the FDR correction (estimate = -1.90 ± 0.67 , $p = 0.017$, FDR $p = 0.038$). Several functional PFAS groups showed associations: amine was positively associated with complement lysis (estimate = 0.84 ± 0.27 , $p = 0.0031$, FDR $p = 0.010$), whereas carboxylic and sulfonic showed no significant effects. All remaining PFAS—including PFBA, PFBS, PFDOA, PFHpA, PFHpS, PFOS, PFPES, total PFAS, chain-long PFAS, Legacy, and next-gen—showed no significant associations (FDR $p > 0.05$). Overall, these results indicate that complement activity was more frequently associated with PFAS exposure than IgY, with predominantly negative exposure-response relationships observed across several long-chain PFAS, alongside strong positive associations for PFHxA and amine.

3.6. PFAS and sex/age interaction analysis

Across the PFAS analytes examined, there was limited evidence that main effects substantially influenced IgY or complement-mediated lysis (Supplemental Table 8). No individual PFAS—including PFBA, PFBS,

PFDA, PFDS, PFDOA, PFHpA, PFHpS, PFNA, PFNS, PFOA, PFOS, PFPES, PFTRdA, PFUNdA, and total PFAS—showed statistically significant main effects on either immune metric after FDR adjustment (all FDR > 0.90). Interactions with sex were uniformly nonsignificant (all FDR ≈ 0.99), indicating no evidence that PFAS exposures differentially affected males and females (Figs. S5 and S6). For IgY, a small negative PFOS-by-age interaction was observed (estimate = -0.011 , $p = 0.03$), but this association did not remain significant after FDR correction (FDR = 0.98). Overall, age was consistently and positively associated with IgY across models (estimates 0.015 – 0.024 per year; all FDR < 0.003).

3.7. Associations between PFAS exposure and white blood cell metrics

Unfortunately, white blood cell counts are not available from the 2024 sampling season. These birds were from the Lake Superior and Lake Michigan regions. As Lake Superior counties were sampled in 2023, we do not suspect major differences in WBC metrics; thus, the main gap is from Lake Michigan counties which tended to have higher proportions of PFUnA and FOSA (Dehnert et al., 2026).

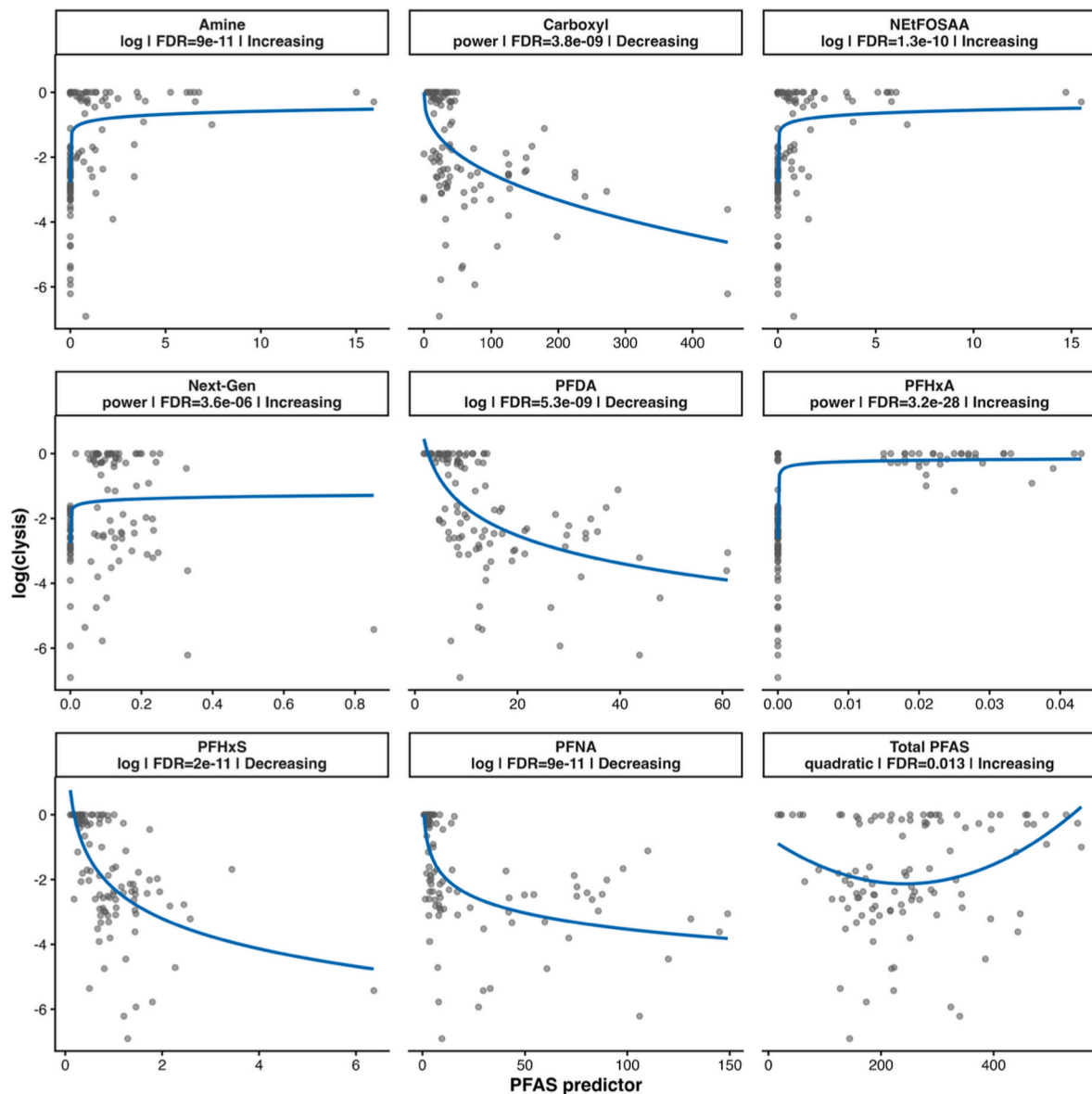


Fig. 3. Key PFAS predictors of cellular immunity (complement lysis) in eagles. Panel show the nine selected PFAS-complement lysis relationships, for the most significant functional groupings and the top-ranked associations by false discovery rate (FDR). Lines represent the best-fit models (linear, log, quadratic, exponential, or power).

After controlling for sex, age, and nest identity, most individual PFAS compounds showed limited associations with WBC proportions and total counts (Fig. S6; Supplemental Table 9). Significant associations after the FDR correction were restricted to heterophil proportions (Fig. 4). Higher concentrations of several structural groupings—including long-chain PFAS (estimate = 0.35 ± 0.10 , $p = 0.0018$, FDR $p = 0.014$), short-chain PFAS (0.33 ± 0.10 , $p = 0.0028$, FDR $p = 0.014$), carboxylates (0.35 ± 0.09 , $p = 0.0006$, FDR $p = 0.014$), sulfonates (0.34 ± 0.10 , $p = 0.0030$, FDR $p = 0.014$), and legacy PFAS (0.35 ± 0.10 , $p = 0.0018$, FDR $p = 0.014$)—were positively associated with heterophil proportions, indicating higher heterophil percentages with greater PFAS exposure. In contrast, PFHxA was negatively associated with the heterophil proportion (estimate = -0.25 ± 0.08 , $p = 0.0040$, FDR $p = 0.015$), indicating a reduction in heterophil proportion with increasing PFHxA concentrations. No other individual PFAS–WBC associations were significant. As PFHxA was not high in Lake Michigan counties (e.g. WBC data from 2024), we do not suspect these missing data would contribute further to our understanding of this relationship.

We observed several additional nominal associations, such as PFNS

was positively associated with heterophil proportion (estimate = 0.19 ± 0.08 , $p = 0.021$, FDR $p = 0.060$). PFHpA showed a positive association with basophil proportion (0.22 ± 0.09 , $p = 0.032$, FDR $p = 0.33$), while PFBA was negatively associated with basophils (-0.23 ± 0.10 , $p = 0.029$, FDR $p = 0.33$). Total WBC counts showed positive trends across several PFAS groupings (estimates ≈ 0.13 – 0.15 , $p = 0.012$ – 0.034), but none were significant after the FDR correction (all FDR $p \geq 0.10$). Eosinophil, lymphocyte, monocyte, and basophil proportions were not correlated to PFAS exposure (Fig. S7). Overall, these results indicate that PFAS exposure was selectively associated with shifts in heterophil proportions—particularly across structural PFAS groupings and inversely with PFHxA—rather than broad alterations in overall leukocyte composition.

4. Discussion

The present study evaluated associations between PFAS exposure and immune biomarkers in bald eagle nestlings sampled across Wisconsin, USA. PFAS are highly persistent environmental contaminants

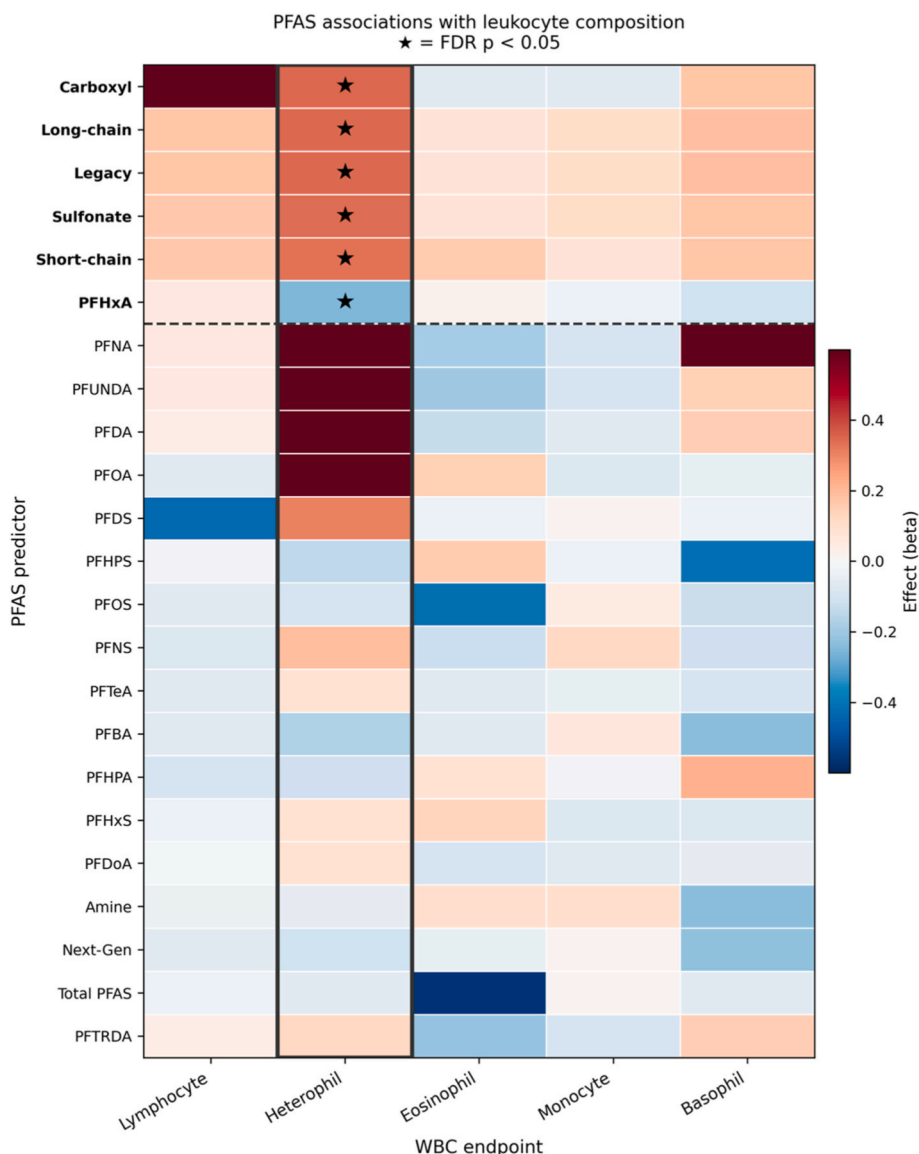


Fig. 4. Heat map of PFAS associations with white blood cell (WBC) endpoints from mixed-effects models. Tile color indicates the model effect estimate (β): red showing positive associations, blue negative associations, and white showing effects near zero. Black dots within the tiles indicate PFAS-WBC associations that remained significant after FDR correction (<0.05). Models were adjusted for age and sex with nest included as a random intercept. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

that are now detected across nearly all ecosystems worldwide (Evich et al., 2022), and exposure in bald eagles reflects their position as apex predators integrating contamination across freshwater food webs. Previous monitoring of these populations has shown substantial spatial variability in PFAS exposure, with several analytes—including PFNA, PFOS, and PFUnA—reaching relatively high concentrations in nestlings from certain regions (>75 ng/mL) (Dehnert et al., 2026). Consistent with findings from human and experimental studies, literature suggests that PFAS can disrupt immune processes (Brand et al., 2025, 2025; Guillette et al., 2022a; Post et al., 2025; von Holst et al., 2021), and our results indicate that PFAS exposure was associated with slight changes in innate immune biomarkers in nestling bald eagles. Associations were compound-specific and heterogeneous, with both positive and negative relationships, future efforts should contextualize these results with regards to immunological protection.

Complement-mediated lysis showed stronger and more frequent associations with PFAS than IgY. The complement system is considered a crucial part of the innate immune system and a first-line defense against pathogens and bridges to adaptive immunity via T-cell and B-cell

signaling (Leslie and Mayor, 2013; Matson et al., 2005). Transcriptomic analyses from human liver cells have reported reduced complement gene expression following PFAS exposure (Addicks et al., 2025). Here, the patterns with PFAS exposure show semi-competing patterns (e.g., decreasing vs. increasing). Importantly, many of the increasing trends (e.g., higher lysis ability with higher PFAS exposure) were weaker (e.g., near-zero slopes), with the exception of PFOS, PFTEA, and sulfonic-PFAS types. Many of the analytes and PFAS categories showed decreasing lysis ability with higher PFAS exposure, which could suggest that PFAS can interfere with lipid metabolism and homeostasis (Corsini et al., 2014; DeWitt et al., 2012; Fenton et al., 2021). Therefore, complement lysis may represent an early physiological indicator of contaminant-associated immune modulation in wildlife. However, further directed experimental studies are needed to tease apart the direction of this relationship and which PFAS are driving these responses, especially in mixtures.

Humoral immunity appeared less sensitive to PFAS exposure than complement immunity. While a few analytes (PFNA, PFDA, PFOA) showed modest positive associations with IgY, slopes were uniformly

shallow and overall the dominant pattern was a negative relationship between IgY and PFAS exposure — particularly for PFOS, sulfonic-PFAS, and total PFAS. This directional pattern is consistent with suppression of humoral immunity documented in laboratory rodents and humans, including reduced IgM titers following PFOS exposure (Peden-Adams et al., 2008) and decreased vaccine antibody responses (Fenton et al., 2021; Phelps et al., 2024).

PFAS exposure was associated with selective shifts in immune cell composition rather than broad changes in total WBC counts. Notably, heterophils — avian first-responders to foreign materials, participating in antimicrobial activity (Harmon, 1998) — generally increased with exposure to carboxylic, sulfonic, long-chain, and legacy PFAS, counter to prior studies reporting reduced or unchanged heterophils in PFAS-exposed birds (Guillette et al., 2022b; Phelps et al., 2024). Eosinophils showed a contrasting pattern, with a notably strong negative association with total PFAS. Other cell types (lymphocytes, monocytes, basophils) showed weaker and more variable responses. The opposing directionality across cell types suggests that PFAS analytes may have antagonistic or class-specific effects on innate cellular immunity, warranting further investigation into mixture interactions.

Several PFAS-immune associations exhibited non-linear exposure–response relationships, with log-linear, quadratic, and power functions frequently providing the best model fit. Until more recently, non-linear toxicological relationships were not considered plausible, however recent literature continues to suggest non-linear toxicological relationships (Lagarde et al., 2015), and our present findings suggest that if we assume strict linear relationships, we may overlook or mischaracterize biologically relevant responses. Non-linear dose–response patterns (e.g., non-monotonic dose responses, thresholds or low-dose non-linearity dose responses, or saturable dose responses) are becoming more widely documented in toxicology studies (Gauthier and Vijayan, 2018; Haney, 2015; Lagarde et al., 2015; Ritz, 2010) and epidemiology studies (May and Bigelow, 2006; Rothenberg and Rothenberg, 2005), where threshold responses or receptor-mediated processes can generate curvilinear exposure–response relationships. Ecotoxicological dose–response analyses frequently employ models such as log-logistic or other non-linear functions to better represent biological responses across exposure gradients (Ritz, 2010). Similar non-linear modeling frameworks have also been used to detect contaminant effects in aquatic organisms, where complex response patterns may not be detectable using linear models alone (Gauthier and Vijayan, 2018). By allowing multiple candidate functional forms rather than assuming linearity *a priori*, our approach provides a more nuanced characterization of potential PFAS-immune relationships in wildlife. These findings highlight the importance of evaluating multiple candidate exposure–response shapes when assessing contaminant effects in wildlife systems.

The above outlined immune changes—particularly in baseline defenses like complement-mediated lysis—may have downstream consequences for disease susceptibility, survival, and ultimately population health. Collectively, our findings demonstrate that sublethal physiological endpoints in eagle nestlings provide a sensitive, spatially explicit signal of environmental contamination, supporting their role as effective biosentinels in freshwater ecosystems. Because these associations are not causal and may reflect co-exposure to other environmental stressors (e.g., other chemicals) or other physiological covariates (e.g., body condition, nutritional status, etc.), future research should explore how different chemical mixtures (both within PFAS and among other contaminants) and other physiological stressors ultimately play a role in wildlife health. Altogether, the consistency of immune responses across a broad geographic scale suggests that PFAS mixtures can drive sublethal physiological changes that may precede population-level effects.

5. Conclusions

Overall, this study serves as a starting point for understanding the immunomodulatory effects of PFAS in free-ranging wildlife. While we

observed several notable trends across multiple components of the immune system (e.g., cellular versus humoral responses), the relationships were complex and varied across analytes and immune markers. Because PFAS exposure in natural systems almost always occurs as mixtures rather than individual compounds, future experimental studies should aim to disentangle the targeted effects of specific PFAS analytes and combinations on immune function and physiology. Additionally, because our analyses were largely conducted at the individual analyte level, potential synergistic or antagonistic interactions among co-occurring PFAS cannot be assessed from these data; mixture modeling approaches would be a valuable next step for characterizing joint exposure effects. In addition, ecotoxicological and immunological studies in wildlife are often constrained by the limited availability of species-specific assays and reagents, meaning that the broader consequences of these physiological changes for morbidity (particularly immunological response to pathogens), survival, or population health remain unclear. Given that several PFAS compounds have been shown to reduce antibody responses to emerging viruses such as influenza A (Post et al., 2025), further work is needed to clarify how contaminant exposure may interact with infectious disease dynamics in wildlife populations. Understanding these interactions will be critical for predicting how persistent contaminants may influence the resilience of wildlife populations facing both chemical pollution and emerging pathogens.

CRedit authorship contribution statement

Emily Cornelius Ruhs: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Sophia Horigan:** Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Sean Strom:** Writing – original draft, Supervision, Investigation, Funding acquisition, Data curation, Conceptualization. **J. Dylan Maddox:** Writing – original draft, Methodology, Investigation. **Felix Grewe:** Writing – original draft, Supervision, Funding acquisition. **Gavin Dehnert:** Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Data curation.

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

During the preparation of this work, the author(s) used Codex and ChatGPT in order to correct code and improve grammar and conciseness. 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.envpol.2026.128760>.

Data availability

Data will be made available on request.

References

- Acevedo-Whitehouse, K., Duffus, A.L.J., 2009. Effects of environmental change on wildlife health. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 364, 3429–3438. <https://doi.org/10.1098/rstb.2009.0128>.
- Addicks, G.C., Rowan-Carroll, A., Leingartner, K., Williams, A., Meier, M.J., Lorusso, L., Yauk, C.L., Atlas, E., 2025. Identification of four mechanisms of toxicity for per- and polyfluoroalkyl substances through transcriptomic profiling in human liver spheroids exposed to 24 PFAS. *Toxicol. Sci.* 207, 161–180. <https://doi.org/10.1093/toxsci/kfaf075>.
- Bolker, B., Robinson, D., Menne, D., Gabry, J., Buerkner, P., Hua, C., Petry, W., Wiley, J., Kennedy, P., Se, E.S., sponsor, B., Patil, I., Arel-Bundock, V., Denney, B., Brunson, C., Wasserman, J., Stukalov, A., Bruneaux, M., 2026. *Broom.Mixed: Tidying Methods for Mixed Models*.
- Bortolotti, G., 2004. 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 & reservoirs: science. Policy and Management for Sustainable Use* 7, 183–187. <https://doi.org/10.1046/j.1440-1770.2002.00186.x>.
- Brand, A.-F., Peterson, S., Günzel, L.M.S., Yap, K.N., Ciesielski, T.M., Arzel, C., Jaspers, V. L.B., 2025. Gene expression changes in ducklings exposed in ovo to emerging and legacy per-/poly-fluoroalkyl substances. *Environ. Toxicol. Chem.* 44, 3477–3491. <https://doi.org/10.1093/etjnl/vgaf213>.
- Brooks, M., Bolker, B., Kristensen, K., Maechler, M., Magnusson, A., McGillicuddy, M., Skaug, H., Nielsen, A., Berg, C., Benthann, K. van, Sadat, N., Lüdecke, D., Lenth, R., O'Brien, J., Geyer, C.J., Jagan, M., Wiernik, B., Stouffer, D.B., Agronah, M., Akdur, H. T.K., Bové, D.S., Krieger, N., Williams, C., 2026. *Glmmtmb: Generalized Linear Mixed Models Using Template Model Builder*.
- 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>.
- Campbell, T., Dein, J., 1984. Avian Hematology. *Veterinary Clinics of North America Small Animal Practice* 14 (2), 223–248. [https://doi.org/10.1016/S0195-5616\(84\)50031-X](https://doi.org/10.1016/S0195-5616(84)50031-X).
- Carson, R., 1962. *Silent Spring*.
- Castano-Ortiz, J.M., Jaspers, V.L.B., Waugh, C.A., 2019. PFOS mediates immunomodulation in an avian cell line that can be mitigated via a virus infection. *BMC Vet. Res.* 15, 214. <https://doi.org/10.1186/s12917-019-1953-2>.
- Chou, T.N., Naumova, E.N., Gass Jr., J.D., 2025. Highly pathogenic avian influenza: a one Health perspective on risks, surveillance, and resilience. *ImmunoHorizons* 9, v1af061. <https://doi.org/10.1093/immhor/v1af061>.
- Cornelius, E.A., Vézina, F., Regimbald, L., Hallot, F., Petit, M., Love, O.P., Karasov, W.H., 2017. Chickadees faced with unpredictable food increase fat reserves but certain components of their immune function decline. *Physiol. Biochem. Zool.* 90, 190–200. <https://doi.org/10.1086/689913>.
- Corsini, E., Luebke, R.W., Germolec, D.R., DeWitt, J.C., 2014. Perfluorinated COMPOUNDS: EMERGING POPs WITH POTENTIAL IMMUNOTOXICITY. *Toxicol. Lett.* 230, 263–270. <https://doi.org/10.1016/j.toxlet.2014.01.038>.
- 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>.
- Custer, C.M., Custer, T.W., Delaney, R., Dummer, P.M., Schultz, S., Karouna-Renier, N., 2019. Perfluoroalkyl contaminant exposure and effects in tree swallows nesting at clarks marsh, oscoda, Michigan, USA. *Arch. Environ. Contam. Toxicol.* 77, 1–13. <https://doi.org/10.1007/s00244-019-00620-1>.
- Custer, C.M., Custer, T.W., Dummer, P.M., Etterson, M.A., Thogmartin, W.E., Wu, Q., Kannan, K., Trowbridge, A., McKann, P.C., 2014. Exposure and effects of perfluoroalkyl substances in tree swallows nesting in Minnesota and Wisconsin, USA. *Arch. Environ. Contam. Toxicol.* 66, 120–138. <https://doi.org/10.1007/s00244-013-9934-0>.
- Custer, C.M., Custer, T.W., Schoenfuss, H.L., Poganski, B.H., Solem, L., 2012. Exposure and effects of perfluoroalkyl compounds on tree swallows nesting at Lake Johanna in east central Minnesota, USA. *Reprod. Toxicol.* 33, 556–562. <https://doi.org/10.1016/j.reprotox.2011.01.005>.
- Custer, T.W., Dummer, P.M., Custer, C.M., Wu, Q., Kannan, K., Trowbridge, A., 2013. Perfluorinated compound concentrations in great blue heron eggs near st. Paul, Minnesota, USA, in 1993 and 2010–2011. *Environ. Toxicol. Chem.* 32, 1077–1083. <https://doi.org/10.1002/etc.2146>.
- 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.* 40, 631–657. <https://doi.org/10.1002/etc.4935>.
- Dehnert, G., Lazarcik, J., Haney, S., Strom, S., Maddox, D., Grewe, F., Cornelius Ruhs, E., 2026. Bald Eagles as bio-sentinels: Monitoring and Fingerprinting PFAS Contamination. <https://doi.org/10.2139/ssrn.6024850>.
- DeLeon, S., Webster, M.S., DeVoogd, T.J., Dhondt, A.A., 2020. Developmental polychlorinated biphenyl exposure influences adult zebra finch reproductive behaviour. *PLoS One* 15, e0230283. <https://doi.org/10.1371/journal.pone.0230283>.
- DeWitt, J.C., Peden-Adams, M.M., Keller, J.M., Germolec, D.R., 2012. Immunotoxicity of perfluorinated compounds: recent developments. *Toxicol. Pathol.* 40, 300–311. <https://doi.org/10.1177/0192623311428473>.
- Dykstra, C.R., Meyer, M.W., Rasmussen, P.W., Keith Warnke, D., 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., 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>.
- Evich, M., Davis, M., McCord, J., Acrey, B., Awkerman, J., Knappe, D., Lindstrom, A., Speth, T., Tebes-Stevens, C., Strynar, M., Wang, Z., Weber, E., Henderson, W., Washington, J., 2022. Per- and Poly Fluoroalkyl substances in the environment. *Science* 375 (6580), eabg9065. <https://doi.org/10.1126/science.abg9065>.
- Fenton, S.E., Ducatman, A., Boobis, A., DeWitt, J.C., Lau, C., Ng, C., Smith, J.S., Roberts, S.M., 2021. Per- and Polyfluoroalkyl substance toxicity and human health review: current State of knowledge and strategies for informing future research. *Environ. Toxicol. Chem.* 40, 606–630. <https://doi.org/10.1002/etc.4890>.
- Fernie, K.J., Mayne, G., Shutt, J.L., Pekarik, C., Grasman, K.A., Letcher, R.J., Drouillard, K., 2005. Evidence of immunomodulation in nestling American kestrels (*Falco sparverius*) exposed to environmentally relevant PBDEs. *Environ Pollut* 138, 485–493. <https://doi.org/10.1016/j.envpol.2005.04.008>.
- Gauthier, P.T., Vijayan, M.M., 2018. Nonlinear mixed-modelling discriminates the effect of chemicals and their mixtures on zebrafish behavior. *Sci. Rep.* 8, 1999. <https://doi.org/10.1038/s41598-018-20112-x>.
- Guillette, T.C., Jackson, T.W., Guillette, M., McCord, J., Belcher, S.M., 2022a. Blood concentrations of per- and polyfluoroalkyl substances are associated with autoimmune-like effects in American alligators from Wilmington, North Carolina. *Front. Toxicol.* 4. <https://doi.org/10.3389/ftox.2022.1010185>.
- Guillette, T.C., Jackson, T.W., Guillette, M., McCord, J., Belcher, S.M., 2022b. Blood concentrations of per- and polyfluoroalkyl substances are associated with autoimmune-like effects in American alligators from Wilmington, North Carolina. *Frontiers in Toxicology* 4.
- Haney, J., 2015. Consideration of non-linear, non-threshold and threshold approaches for assessing the carcinogenicity of oral exposure to hexavalent chromium. *Regul. Toxicol. Pharmacol.* 73, 834–852. <https://doi.org/10.1016/j.yrtph.2015.10.011>.
- Harmon, B., 1998. Avian heterophils in inflammation and disease resistance. *Poult. Sci.* 77, 972–977. <https://doi.org/10.1093/ps/77.7.972>.
- Hellgren, O., Waldenström, J., Bensch, S., 2004. A new PCR assay for simultaneous studies of Leucocytozoon, Plasmodium, and Haemoproteus from avian blood. *J. Parasitol.* 90 (4), 797–802. <https://doi.org/10.1645/GE-184R1>.
- Karouna-Renier, N.K., Haskins, D.L., Schultz, S.L., Akresh, M.E., Rattner, B.A., 2026. Accumulation of per- and polyfluoroalkyl substances (PFAS) and their association with immune parameters in nestling ospreys (*Pandion haliaetus*) from Chesapeake and Delaware Bays, USA. *Environmental Toxicology and Chemistry* vgag055. <https://doi.org/10.1093/etjnl/vgag055>.
- Killpack, T.L., Carrel, E., Karasov, W.H., 2015. Impacts of short-term food restriction on immune development in altricial house sparrow nestlings. *Physiol. Biochem. Zool.* Ecological and Evolutionary Approaches 88, 195–207. <https://doi.org/10.1086/680168>.
- 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.: Processes Impacts* 21, 1887–1898. <https://doi.org/10.1039/C9EM00281B>.
- Lagarde, F., Beausoleil, C., Belcher, S.M., Belzunces, L.P., Emond, C., Guerbet, M., Rousselle, C., 2015. Non-monotonic dose-response relationships and endocrine disruptors: a qualitative method of assessment. *Environ. Health* 14, 13. <https://doi.org/10.1186/1476-069X-14-13>.
- 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>.
- Leslie, J.D., Mayor, R., 2013. Complement in animal development: unexpected roles of a highly conserved pathway. *Semin. Immunol.* 25, 39–46. <https://doi.org/10.1016/j.smim.2013.04.005>.
- Lochmiller, R.L., Deerenberg, C., 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* 88, 87–98. <https://doi.org/10.1034/j.1600-0706.2000.880110.x>.
- Maddox, J.D., Feldheim, K.A., 2014. A cost-effective size standard for fragment analysis that maximizes throughput on five dye set platforms. *Conservation Genet Resour* 6, 5–7. <https://doi.org/10.1007/s12686-013-0019-1>.
- Matson, K.D., Ricklefs, R.E., Klasing, K.C., 2005. A hemolysis-hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. *Dev. Comp. Immunol.* 29, 275–286. <https://doi.org/10.1016/j.dci.2004.07.006>.

- May, S., Bigelow, C., 2006. Modeling nonlinear dose-response relationships in epidemiologic studies: statistical approaches and practical challenges. *Dose Response* 3, 474–490. <https://doi.org/10.2203/dose-response.003.04.004>.
- Merchant, M., Hammack, T., Sanders, P., Dronette, J., 2006. Rapid and inexpensive method for the spectroscopic determination of innate immune activity of crocodilians. *Spectrosc. Lett.* 39, 337–343. <https://doi.org/10.1080/00387010600781290>.
- Owen, J.C., 2011. Collecting, processing, and storing avian blood: a review. *J. Field Ornithol.* 82, 339–354. <https://doi.org/10.1111/j.1557-9263.2011.00338.x>.
- Peden-Adams, M.M., Keller, J.M., Eudaly, J.G., Berger, J., Gilkeson, G.S., Keil, D.E., 2008. Suppression of humoral immunity in mice following exposure to perfluorooctane sulfonate. *Toxicol. Sci.* 104, 144–154. <https://doi.org/10.1093/toxsci/kfn059>.
- Phelps, D.W., Connors, A.M., Ferrero, G., DeWitt, J.C., Yoder, J.A., 2024. Per- and polyfluoroalkyl substances alter innate immune function: evidence and data gaps. *J. Immunotoxicol.* 21, 2343362. <https://doi.org/10.1080/1547691X.2024.2343362>.
- Podder, A., Sadmani, A.H.M.A., Reinhart, D., Chang, N.-B., Goel, R., 2021. Per and polyfluoroalkyl substances (PFAS) as a contaminant of emerging concern in surface water: a transboundary review of their occurrences and toxicity effects. *J. Hazard Mater.* 419, 126361. <https://doi.org/10.1016/j.jhazmat.2021.126361>.
- Post, C.M., Agyemang, J.J., McDonald, K.A., McDonough, C.A., Lawrence, B.P., 2025. Developmental exposure to a PFAS mixture impairs the anamnestic response to influenza A virus infection in mice. <https://doi.org/10.1101/2025.11.10.687657>.
- R Core Team, 2024. *R: a Language and Environment for Statistical Computing*.
- Ritz, C., 2010. Toward a unified approach to dose–response modeling in ecotoxicology. *Environ. Toxicol. Chem.* 29, 220–229. <https://doi.org/10.1002/etc.7>.
- Robuck, A.R., Cantwell, M.G., McCord, J., Addison, L.M., Pfohl, M., Strynar, M.J., McKinney, R., Katz, D.R., Wiley, D.N., Lohmann, R., 2020. Legacy and novel Per- and Polyfluoroalkyl Substances (PFAS) in juvenile seabirds from the US Atlantic Coast. *Environ. Sci. Technol.* 54, 12938–12948. <https://doi.org/10.1021/acs.est.0c01951>.
- Ross, T.A., Zhang, J., Wille, M., Asimakopoulou, A.G., Jaspers, V.L.B., Klaassen, M., 2025. Interplay between pollution and Avian influenza virus in shorebirds and waterfowl. *EcoHealth* 22, 222–232. <https://doi.org/10.1007/s10393-025-01707-z>.
- Rothenberg, S.J., Rothenberg, J.C., 2005. Testing the dose–response specification in epidemiology: public health and policy consequences for lead. *Environ. Health Perspect.* 113, 1190–1195. <https://doi.org/10.1289/ehp.7691>.
- Route, W.T., Russell, R.E., Lindstrom, A.B., Strynar, M.J., Key, R.L., 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>.
- Sehgal, R., Hull, A., Anderson, N., Valkiunas, G., Markovets, M., Kawamura, S., Tell, L., 2006. Evidence for cryptic speciation of *Leucocystozoospora* spp. (Haemosporida, Leucocytozoidae) in diurnal raptors. *J. Parasitol.* 92, 375–379. <https://doi.org/10.1645/GE-656R.1>.
- Sheldon, B.C., Verhulst, S., 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends Ecol. Evol.* 11, 317–321. [https://doi.org/10.1016/0169-5347\(96\)10039-2](https://doi.org/10.1016/0169-5347(96)10039-2).
- Strom, S., Wojcik, B., Dehnert, G., Cornelius Ruhs, E., 2025. Longitudinal trends of Per- and polyfluoroalkyl substances (Pfas) in nestling bald eagles (*Haliaeetus leucocephalus*) from Wisconsin. *Environ. Res.* 276. <https://doi.org/10.1016/j.envres.2025.121468>.
- US EPA, 2021. Per- and Polyfluoroalkyl Substances (PFAS) [WWW Document]. URL. <https://www.epa.gov/sdwa/and-polyfluoroalkyl-substances-pfas>, 3.13.26.
- USEPA, 2024. Method 1633, Revision A Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS.
- von Holst, H., Nayak, P., Dembek, Z., Buehler, S., Echeverria, D., Fallacara, D., John, L., 2021. Perfluoroalkyl substances exposure and immunity, allergic response, infection, and asthma in children: review of epidemiologic studies. *Heliyon* 7, e08160. <https://doi.org/10.1016/j.heliyon.2021.e08160>.
- Waldenström, J., Bensch, S., Hasselquist, D., Ostman, O., 2004. A new nested polymerase chain reaction method very efficient in detecting plasmodium and haemoproteus infections from avian blood. *J. Parasitol.* 90 (1). <https://doi.org/10.1645/GE-3221RN>, 191–4.
- Wells, M.R., Coggan, T.L., Stevenson, G., Singh, N., Askeland, M., Lea, M.-A., Philips, A., Carver, S., 2024. Per- and polyfluoroalkyl substances (PFAS) in little penguins and associations with urbanisation and health parameters. *Sci. Total Environ.* 912, 169084. <https://doi.org/10.1016/j.scitotenv.2023.169084>.
- WI DNR, 2022. 2022 PFAS Maximum Contaminant Levels | NR 809 SAFE DRINKING WATER STANDARDS UPDATE [WWW Document]. URL. <https://dnr.wisconsin.gov/topic/DrinkingWater/2022PFASMCs.html>, 3.13.26.