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Tracking early-life PFAS exposure in the Ebro Delta flamingo colony: temporal trends and sources

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ABSTRACT

The Greater flamingo (*Phoenicopterus roseus*) is one of the most recognizable species breeding in the Ebro Delta Natural Park. In 2019, high per- and polyfluoroalkyl substances (PFAS) concentrations were found in two months old flamingo chicks. Motivated by these findings and the global concern for PFAS contamination, we monitored flamingos PFAS exposure over a five-year period. The study aimed to assess PFAS contamination patterns in chick's blood, temporal trends, examine potential effects on their body condition, and identify potential contamination sources by analysing PFAS in water. We collected a total of 148 flamingo blood samples from 2019 (n = 47), 2022 (n = 51), and 2024 (n = 50), and water samples (n = 8) within the Natural Park.

All chicks from the three studied years presented PFAS in their blood, indicating a chronic and early-life exposure to these contaminants to the breeding colony. ∑PFAS concentrations ranged from 4.53 to 576 ng/mL, and PFOS, PFOA and PFHxS were detected in all individuals. In 2024, mean ∑PFAS were of 55 ng/mL, lower than those detected in 2019 (78.2 ng/mL) and 2022 (81.7 ng/mL) ($p < 0.05$). The PFAS decline was driven by lower PFOA and PFHxS levels, likely reflecting recent European regulatory measures. The body condition of flamingos was found to be higher in those years with higher total precipitation during the breeding season ($p < 0.05$), likely due to a higher productivity of feeding sites. Notably, individuals with higher PFHxS and PFHpA concentrations exhibited reduced body condition ($p < 0.05$). PFAS were detected in all water samples indicating widespread contamination in the area, with the highest concentrations in agricultural fields which are important flamingo feeding areas. Overall, this study provides evidence of early PFAS exposure in a Special Protection Area for Birds and demonstrates the value of incorporating contaminant biomonitoring into conservation programs.

1. Introduction

The Ebro Delta is a natural area located in the western Mediterranean at the end of the Ebro River. Covering approximately 330 km^2, it hosts over 400 bird taxa, of which nearly one hundred species breed regularly (Bigas and Curcó, 2024). The Ebro Delta is internationally recognized for its ecological richness and its relevance for waterbird conservation, it is designated as an Important Bird and Biodiversity area (IBA) by Birdlife International (BirdLife International, 2026a,b) and it is listed in a Ramsar Wetland of International Importance (RAMSAR, 2025). Since 1983, the Ebro Delta is a Natural Park (EDNP) included in the Natura 2000 Network as a Special Protection Area for Birds (SPA), underscoring its critical importance for biodiversity conservation.

Anthropogenic activities represent a constant source of organic pollutants input into this natural and fragile area. Several studies have identified the presence of persistent organic pollutants (POPs) and per- and polyfluoroalkyl substances (PFAS) in water and sediments (Čelić et al., 2019; Gómez-Gutiérrez et al., 2006; Pignotti et al., 2017) and wildlife (Ayala-Cabrera et al., 2021; Bertolero et al., 2015; Gómez-Gutiérrez et al., 2006; Pignotti et al., 2017). These contaminants pose a significant threat to wildlife due to their persistence, bioaccumulation potential, and toxicity.

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Greater flamingos (*Phoenicopterus roseus*) are one of the most recognizable and best-studied birds in the Ebro Delta. The staff at the Natural Park have monitored the number of nesting pairs for more than 30 years, and since 2004 the chicks have also been ringed. Flamingos are very vulnerable to habitat loss, extreme weather events, predation, human disturbance, and pollution (Delfino and Carlos, 2024; Mooney et al., 2023). Climate change, together with the decline of wetlands areas, pose at risk the stability of flamingo populations, raising the need to combine efforts from science and policymakers to create management plans to prevent the future decline of the species (Delfino, 2023).

The threat of chemical pollution to flamingo conservation is still poorly studied. There are few studies reporting flamingo exposure to mostly heavy metals (Ancora et al., 2008; Gillingham et al., 2021; Rocha et al., 2021; Valiente et al., 2022) and legacy organochlorine pesticides (Guitart et al., 2005). Until a few years ago, the impact of environmental pollutants on the Ebro Delta flamingo population was virtually unknown. In 2019, the first contaminant analysis in flamingo chicks from the Ebro Delta was conducted to assess the breeding colony's exposure to environmental contamination. Nearly one hundred compounds from different chemical families were examined: organochlorine pesticides (OCPs), polychlorinated biphenyl (PCBs), polycyclic aromatic hydrocarbons (PAHs), pharmaceuticals, and PFAS. Among all the contaminant families analysed, PFAS were not only detected in every individual but also found at the highest concentrations. Specifically, PFAS mean concentrations (78.2 ± 12 ng/mL) nearly doubled those of PAHs (31 ± 8.9 ng/mL) and were an order of magnitude higher than OCPs (8.58 ± 1.3 ng/mL) and PCBs (2.3 ± 0.5 ng/mL) (Dulsat-Masvidal et al., 2023). These results are particularly striking, as PFAS concentrations clearly exceeded those of all the other contaminant families analysed, raising concern about potential chronic PFAS exposure within the colony.

PFAS are a large chemical family, with more than seven million compounds identified, commonly classified as carboxylic acids (-COOH) (PFCAs) and sulfonic acids (-SO_3H) (PFSAs) (Schymanski et al., 2023). Their high persistence and resistance to degradation, together with their extensive use in industrial and consumer products, have led to their widespread detection in the environment, earning them the label of "forever chemicals".

The findings from 2019, and the growing global interest in PFAS contamination, bring out the question of how the breeding colony of flamingos were exposed to PFAS and to which extent this exposure was chronically persistent in the area. However, exceptional circumstances delayed the continuation of sampling: in 2020 and 2021, the COVID-19 pandemic hampered ringing logistics, and in 2023, the avian influenza outbreak posed a high risk for handling wild birds. Finally, flamingo ringing activities in the Natural Park proceeded in 2022 and 2024, allowing the collection of blood samples. In 2025, flamingos were not ringed due to high reproductive failure (0.21 chicks/pairs; EDNP pers. comm). Part of this failure may have been caused by predation and disturbance by the Yellow-legged Gull (*Larus michahellis*), but other factors may also have affected the colony.

In this study we have analyzed PFAS in blood in 2-month old flamingo chicks from the Ebro Delta with the aim to I) determine the PFAS exposure over a five-year period (2019 to 2024); II) assess changes in the body condition of flamingo chicks through the three sampling periods associated with PFAS exposure; and III) identify potential sources of PFAS within this protected natural area by monitoring their presence in waters collected in representative habitats used by flamingos.

2. Material and methods

2.1. Reagents and standards

All PFAS standards were purchased from Wellington Laboratories (Guelph, Ontario, Canada). Standards used were the PFAC-MXH native mixture solution containing 25 PFAS, and the MPFAC-MXA mixture solution, containing 9 isotopically labelled standards, used as internal standards (IS). The list of target analytical standards and IS provided in Table S1.

2.2. Sample collection

Blood samples were obtained during the annual ringing activity organized by the Ebro Delta Natural Park. The breeding colony was located at 40° 34′ 29.04″ N, 0° 40′ 17.15″ E (Fig. 1). The ringing sessions enabled the safe capture of 400 chicks, which were subsequently ringed and measured. At the time of sampling, chicks were approximately two months old, had not yet developed the capacity to fly and gathered in *crèches*, which facilitated their capture. Flamingos exhibit a synchronous nesting within the colony, meaning that all sampled individuals were of a similar age, minimizing age-related variability in the study. For each individual, biometric data were recorded, including body mass (±50 g), wing length, tarsus length (±1 mm), and bill or culmen length (±0.1 mm). Biometric measurements were used to calculate the Scaled Mass Index (SMI), following the method proposed by Peig and Green (2009). SMI was used as a proxy for body condition, as it provides a standardized body mass for each individual independent of their size. This is important at this development stage, where chicks with only a few days of age difference may already exhibit significant variations in size or weight due to their fast growth.

In each ringing campaign, a small group of chicks were randomly selected for blood sampling by veterinary personnel. About 1 mL of blood was drawn from the tarsal vein and stored in 2 mL Eppendorf tubes (Eppendorf, Hamburg, Germany) containing heparin and labelled with the individual ringing code. Samples were kept refrigerated in the field and later stored at −21 °C. Sample collection followed the good practice guidelines described by Espín et al. (2021). A total of 148 blood samples from 2019 (n = 47), 2022 (n = 51), and 2024 (n = 50) were obtained. All procedures were authorized by the Department of Climate Action, Food and Rural Agenda of the Generalitat de Catalunya, and permits for animal handling and sampling were granted (available upon request).

To determine potential PFAS sources, water samples were collected in 2022 from eight strategic locations (Fig. 1) across both sides of the Ebro River, prioritizing flamingo foraging habitats and surrounding areas within the EDNP. These included agricultural rice fields (site 1 and 3), natural wetlands and saltmarshes (site 4, 6, 7, and 8), and an artificial wetland designed to clean water from agricultural runoffs (site 5). Additionally, a sample from the Ebro River's main flow (site 2) was included to account for external PFAS input to the natural area (Fig. 1).

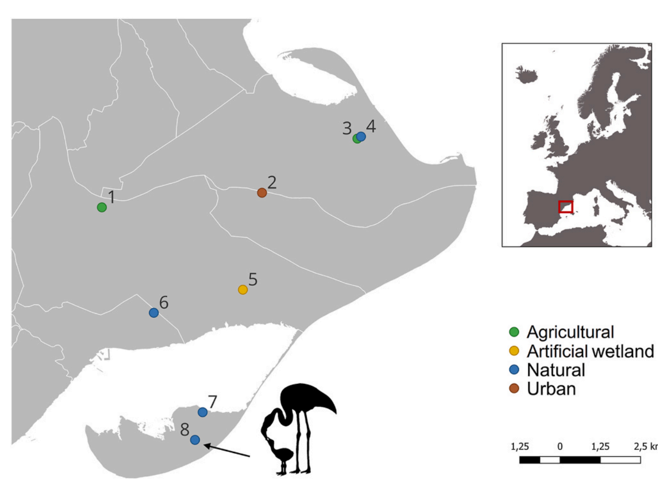

Fig. 1. Map of the Ebro Delta Natural Park indicating the location of the breeding colony of flamingos (arrow) and the water sampling points are indicated in dots (from 1 to 8), indicating in colours the land use.

Samples were collected using a telescope beaker scoop and placed in 1 L amber crystal bottles and kept refrigerated for transportation. Samples were analysed in less than 72 h after collection.

Precipitation data were obtained from the *els Alfacs* automatic meteorological station provided by Meteorological Service of Catalonia (Meteorological Service of Catalonia, 2025). Total precipitation values were compiled for the period between 1st of March and 31st of July in 2019, 2022 and 2024.

2.3. PFAS analysis and method validation

The extraction of PFAS from blood samples was performed using the same protocol described in (Dulsat-Masvidal et al., 2023). Briefly, 100 μL of whole blood was transferred to a 2 mL polypropylene Eppendorf vial (Eppendorf, Hamburg, Germany) and fortified with 10 ng of IS mixture. 1.5 mL of acetonitrile was then added. Samples were vortexed for 1 min and extracted in an ultrasonic bath for 10 min; this cycle was repeated three times without changing the solvent, followed by centrifugation at 3500 rpm for 10 min. The supernatant was collected, and 25 mg of activated carbon and 50 μL of glacial acetic acid were added. The mixture was vortexed for 1 min and centrifuged again for 10 min, then filtered through a 13 mm × 0.2 μm nylon syringe filter (Clarify, Phenomenex, USA). Finally, the extracts were evaporated to dryness with a ReactiVap® (Pierce, Gemini Lab, Apeldoorn, The Netherlands) and reconstituted with 100 μL of methanol and 100 μL of HPLC water with 10 mM ammonium acetate(aq) solution.

Water samples were analysed following the protocol detailed in Dulsat-Masvidal et al. (2022). Two hundred mL of water was spiked with 25 ng of IS mixture, and extracted by solid-phase extraction (SPE) using Oasis HLB 200 mg, 6 cc cartridges (Waters, Mildford, USA). Following preconcentration, the SPE cartridges were dried under vacuum and eluted sequentially with 15 mL of methanol and 6 mL of acetone. The combined extracts were reduced to 0.5 mL using a TurboVap® LV system (Caliper Lifesciences, Uppsala, Sweden) under a gentle nitrogen stream at 25 °C and transferred to a 1.5 mL chromatographic vial using 1 mL of methanol to rinse the container. The samples were then evaporated to dryness and reconstituted in the same way as blood extracts.

The PFAS analysis was conducted by ultra-high-performance liquid chromatography coupled to a Bruker Impact II Q-TOF mass spectrometer (Bruker, Germany) (UHPLC-Q-TOF), following the method developed by Oró-Nolla et al. (2023). A Phenomenex C_{18} Luna Omega column (100 mm length × 2.1 mm inner diameter, 100 Å pore size, 1.6 μm particle size) was used at 40 °C, with an XBridge C_{18} column (50 mm × 4.6 mm, 3.5 μm particle size) used as a trap column to remove background PFAS contribution from the mobile phase and LC tubing. The mobile phase consisted of a binary mixture of (A) methanol and acetonitrile (80:20, v/v) with 10 mM of ammonium acetate and (B) water with 10 mM of ammonium acetate.

Further details on the instrumental protocol and method validation are described in Oró-Nolla et al. (2023). Calibration curves were built from 1 to 300 ng/mL, except for PFBA, 4:2FTS, 6:2FTS and 8:2FTS (4–1200 ng/mL), and PFPeA (2–600 ng/mL) with internal standard (10 ng/mL). PFAS were quantified with the internal standard procedure. Instrumental detection limits (IDL) were calculated from the lowest calibration standard using a signal-to-noise ratio of 3. Instrumental repeatability and reproducibility were determined by injecting a 50 ng/mL standard solution 5 consecutive times and in 5 different days, respectively. The recoveries of the target compounds were assessed by fortifying quail blood and MilliQ water with 10 and 50 ng of native standards, respectively, and 10 and 25 ng of IS. Method detection limits (MDL) were determined from fortified samples using a signal/noise ratio of 3. One to three procedural blanks without matrix and spiked with 10 ng of internal standard were included in each sample batch. All blanks showed levels below the MDL. Solvent blanks were injected every five samples during the instrumental analysis to check and prevent potential carry-over concentrations throughout the LC-QTOF sequence. Quality assurance parameters including recoveries and MDL for blood and water samples are provided in Table S2.

2.4. Data analysis

Data analysis was performed only for compounds with detection frequencies above 55% of the total 148 flamingo individuals. Values below MDL were estimated for compounds detected in more than 55% but less than 100% of the samples using Regression on Order Statistics (ROS), implemented in the Non-detects and Data Analysis for Environmental Data (NADA) package in R (Lee and Helsel, 2025).

PFAS values were left-skewed according to visual inspections of histograms and the Shapiro-Wilk test, accordingly, concentrations were log-transformed prior to the analysis to meet normality assumptions.

Temporal trends were assessed by calculating linear regressions for each compound across sampling years (2019, 2022 and 2024). The contribution of each compound to the overall trend was calculated by computing its mean proportion, calculated as the ratio between the mean concentration of each compound across all samples and the total mean of $\sum$PFAS. Two-way ANOVA followed by Turkey's HSD post-hoc test were used for the comparison of mean concentrations between years. The level of significance was set at p-value 0.05.

To assess the effect of PFAS exposure on the flamingo body condition (SMI), we fitted linear mixed-effects models (LMM) including year as a random effect and total precipitation during the breeding season (March to July) and only the PFAS detected in >55% of the samples as a fixed effect. All predictors were scaled before fitting the models. Four different models were tested following the parsimonious approach (Table S3). The best fitting-model was selected based on the Akaike Information Criteria (AIC), corresponding to the full model that included the six PFAS detected in the samples (PFOS, PFOA, PFHxS, PFNA, PFHpA, PFDA) and the total precipitation. Variance Inflation Factors (VIF) were calculated to assess multicollinearity among the predictors included in the model. All VIF values for all predictors were below 5, indicating low multicollinearity.

PFAS fingerprints in blood and water were calculated for each sample as the relative contribution (%) of each compound to the total sum of PFAS in that sample. Principal Component Analysis (PCA) was then performed to compare the fingerprints between the two matrices. A second PFAS fingerprint was generated considering only compounds detected in more than 55% of flamingo chick blood from 2022. Relative contributions of these compounds were calculated for both blood and water samples. The blood PFAS profiles were compared to the water PFAS profiles from the eight sampled locations using Spearman's rank correlations. The level of significance was set at p-value 0.05.

3. Results and discussion

3.1. PFAS in the flamingo colony through the years

PFAS were detected in all sampled flamingo chicks across the 2019-2022-2024 monitoring campaigns (n = 148), indicating an ongoing exposure of the colony to these contaminants. The detection frequency and concentrations for all individual compounds are detailed in Table 1. Fourteen out of 25 compounds were detected. PFOA, PFOS, and PFHxS were identified as the most prevalent ones, as they were found in all individuals from the three different years. Other frequently detected compounds included PFNA, above 90% of individuals, as well as PFDA and PFHpA, both found in more than 50% of the samples. Other compounds detected were PFBA, PFBS, PFHxA PFHpS, FOSA, PFUdA, PFDoA, PFTrDA, PFTeDA, their concentrations are reported in Table 1.

The mean concentration of $\sum$PFAS found in 2019 was 78.2 ng/mL (from 9.34 to 576 ng/mL) (Table 1), PFOA was detected at the highest concentration (38.5 ± 8.26 ng/mL) and represented the 49% of $\sum$PFAS, followed by PFOS (14.6 ± 0.99 ng/mL) and PFHxS (12.3 ± 1.36 ng/mL). Three years later, in 2022, the colony presented similar levels of $\sum$PFAS

Table 1
PFAS analysed in blood samples of Greater flamingo chicks from Ebro Delta, indicating frequency of detection (f.d., percentage), mean concentration and standard error (S.E.), and range of concentrations, expressed as ng/mL. Compounds analysed but not detected indicated as n.d, whereas compounds not analysed are indicated with n.a. * indicates mean calculated with ROS. Only mean values are provided for compounds detected ≥ 55% of the samples, "-" indicates compounds detected in < 55% of samples. PFAS are ordered by carbon chain length.

Compound	2019 (N = 47)			2022 (N = 51)			2024 (N = 50)		
	f.d. (%)	Mean ± S.E.	Range min – max	f.d. (%)	Mean ± S.E.	Range min - max	f.d. (%)	Mean ± S.E.	Range min - max
PFBA	2	-	0.20 - 0.20	8	-	1.81 - 6.07	6	-	4.55 - 155
PFBS	2	-	1.06 - 1.06	n.d.	n.d.	n.d.	40	-	0.59 - 1.87
PFPeA	n.a.	-	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFPeS	n.a.	-	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHxA	n.d.	n.d.	n.d.	2	-	15.6 - 15.6	48	-	2.24 - 91.7
PFHxS	100	12.3 ± 1.36	1.8 – 39.0	100	16.5 ± 2.00	1.52 - 69.3	100	4.06 ± 0.46	0.70 - 17.1
4:2 FTS	n.a.	-	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHpA	47	-	1.68 - 122	76	4.38 ± 0.59*	0.68 - 20.5	54	1.88 ± 0.28*	0.76 - 12.6
PFHpS	n.a.	-	-	65	1.51 ± 0.15*	0.48 - 5.11	32	-	0.49 - 2.79
PFOA	100	38.5 ± 8.26	3.04 - 366	100	26.3 ± 3.82	1.83 - 140	100	12.2 ± 1.36	1.37 - 40.8
PFOS	100	14.6 ± 0.99	3.83 - 35.5	100	18.2 ± 2.52	1.30 - 85.5	100	14.7 ± 2.15	1.48 - 67.4
FOSA	n.a.	-	-	23	-	0.39 - 3.90	10	-	0.43 - 0.76
NMeFOSAA	n.a.	-	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
NEtFOSAA	n.a.	-	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6:2 FTS	n.a.	-	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFNA	94	5.13 ± 0.67*	0.13 - 24.8	98	11.8 ± 1.62*	1.58 - 62.8	92	4.60 ± 0.59*	0.88 - 28.3
PFNS	n.a.	-	-	n.d.	n.d.	0 - 0	n.d.	n.d.	n.d.
PFDA	74	2.06 ± 0.12*	1.26 - 5.45	71	2.94 ± 0.44*	1.08 – 18.0	64	1.81 ± 0.22*	0.99 - 11.1
PFDS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0	n.d.	n.d.
8:2 FTS	n.a.	-	-	n.d.	n.d.	n.d.	0	n.d.	n.d.
PFUdA	30	-	0.16 - 2.41	27	-	0.80 - 8.45	46	-	0.78 - 5.06
PFDoA	n.d.	n.d.	n.d.	20	-	0.58 - 3.65	44	-	0.58 - 1.81
PFDoS	n.a.	-	-	n.d.	n.d.	n.d.	0	n.d.	n.d.
PFTrDA	40	-	1.31 - 2.63	n.d.	n.d.	n.d.	54	-	0.57 - 2.35
PFTeDA	n.d.	n.d.	n.d.	37	-	0.93 - 11.9	26	-	0.43 - 2.23
∑PFAS	**100**	**78.2 ± 12.5**	**9.34–576**	**100**	**81.7 ± 9.3**	**5.63–349**	**100**	**55.3 ± 7.3**	**4.53–271**

with a mean of 81.7 ng/mL (from 5.63 to 349 ng/mL), the profile of compounds detected also remained very similar with PFOA as the compound detected at the highest concentration (26.3 ± 3.82 ng/mL) representing a contribution of 32.2% of ∑PFAS, followed by PFOS (18.2 ± 2.52 ng/mL) and PFHxS (16.5 ± 2.00 ng/mL). However, in 2024, ∑PFAS concentrations declined significantly ($p < 0.05$), with a mean of 55.3 ng/mL (from 4.53 to 271 ng/mL). The compound detected at the highest concentration was in this case PFOS (14.7 ± 2.15 ng/mL) with a total contribution of 26.6% closely followed by PFOA (12.2 ± 1.36 ng/mL) with a contribution of 22% and PFHxS (4.06 ± 0.46 ng/mL) representing 7.41% of the total PFAS. Although their overall contribution to total PFAS remains low, it is worth to note the increase in the detection frequency of short-chain compounds, such as PFBA and PFHxA. Both compounds were scarcely detected in 2019, but reached detection frequencies of 6% and 48% in the individuals in 2024, respectively. This shift likely reflects the transition towards short-chain PFAS as substitutes of regulated long-chain PFAS, as PFOA (Brendel et al., 2018).

Linear models were generated for the six compounds detected above 55% of the samples through the years to identify which compounds contributed the most to the decline of ∑PFAS observed in 2024 (Fig. 2). The proportion mean is indicative of the relative weight of each compound to the general trend of PFAS, and the slope of the tendency of the compounds along the 5 years period. The compounds that presented a higher contribution to the total mean are indicated in yellow and green colours in Fig. 2. The compounds with the higher negative trend over the years are PFOA, PFHxS and PFHpS. PFOA and PFHxS have mean proportion of 0.235 and 0.178, respectively therefore, they represent the highest contribution to the general trend. Although PFHpS also presents a significant negative decline, its contribution to the total mean is very low, and it cannot explain the total PFAS decline observed in 2024.

PFOS also presents a mean proportion of 0.22, as it is one of the compounds with the highest contribution to the total PFAS; however, the decline of PFOS is not significant ($p > 0.05$). In fact, PFOS concentrations exhibited minimal temporal variation across the five-year sampling period (Table 1). Given that PFOS was listed in the Stockholm Convention in 2009 and has been regulated in the EU since 2011 (EU 207/2011, 2011) (European Commission, 2011), its steady concentration is more likely attributable to its well-documented environmental persistence rather than to ongoing emissions in the study area. In addition to legacy contamination, precursor compounds such as FOSA, NMeFOSAA, and NEtFOSAA can degrade to form PFOS (Martin et al., 2010). In the present study, only FOSA was detected in flamingos' blood, with concentrations ranging from 0.39 to 3.90 ng/mL (Table 1), whereas NMeFOSAA and NEtFOSAA were not detected.

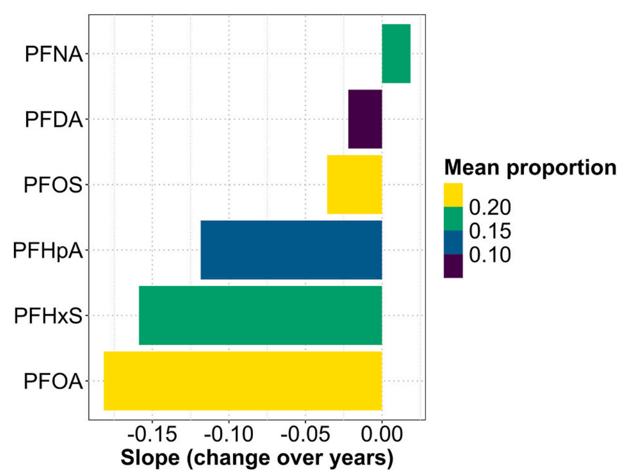

Fig. 2. Trend (slope) of PFAS detected in more than 55% of the samples over the years 2019, 2022 and 2024. The mean proportion was calculated as the average contribution of each compound relative to the ∑PFAS. Colours indicate the relative contribution: yellow and green highlight compounds with a higher mean proportion, reflecting a stronger contribution to the total PFAS trend.

In contrast, PFOA and PFHxS, whose use was regulated under the EU Regulation on persistent organic pollutants in 2020 (EU, 2020/784, 2020) (European commission, 2020) and 2023 (EU, 2023/1608, 2023) (European Commission, 2023), respectively, displayed temporal trends more consistent with recent usage and subsequent regulatory phase-out. These compounds were also added to the Stockholm Convention during the course of this study, PFOA in 2019 and PFHxS in 2022 (Stockholm Convention, 2026).

Mean PFOA concentrations already declined in 2022 (mean of 26.3 ng/mL, Table 1), although this reduction was not statistically significant. PFHxS concentrations remained stable from 2019 to 2022 but exhibited a statistically significant decline in 2024 ($p < 0.05$). These results suggest that the presence of PFOA and PFHxS in flamingos is more closely linked to contemporary sources, and that their decreasing concentrations may reflect the effectiveness of recent regulatory actions and their progressive removal from the market. Similar temporal declines in PFAS concentrations have been reported in other wildlife monitoring programs following regulatory interventions (Badry et al., 2025), supporting the interpretation that reduced environmental inputs drive these trends.

In contrast to most previous studies, in which PFOA is not reported as a dominant compound, the present study identifies PFOA as one of the most prevalent contaminants over the years. The PFOA concentrations detected in 2019 and 2022 are comparable to those reported in European Shags (*Phalacrocorax aristotelis*; 20.9 ± 1.23 ng/mL) in Scotland (Carravieri et al., 2020) and in Tree Swallows (*Tachycineta bicolor*; 17.8 ± 3.65 ng/mL) breeding in an area impacted by firefighting foams (Custer et al., 2019). However, they are approximately three times higher than those found in Lesser Black-backed Gull (*Larus fuscus*) chicks in the Netherlands (7.60 ± 1.36 ng/mL; Lopez-Antia et al. (2021)) and much higher than those reported in top predators such as the Bald Eagle (*Haliaeetus leucocephalus*) in the United States (1.00 ± 0.26 and 2.20 ± 1.79 ng/mL; Dykstra et al. (2021)), Peregrine Falcon (*Falco peregrinus*) in Canada (0.54 ± 0.081 ng/mL and 1.11 ± 0.10 ng/mL; Sun et al. (2020)) and White-tailed Eagle (*Haliaeetus albicilla*) in Norway (0.22 ± 0.02 ng/mL; Løseth et al. (2019)). Also, it is noteworthy that PFOA was not detected in previous studies using gull eggs from the Ebro Delta in the same breeding area (Colomer-Vidal et al., 2022), suggesting that the flamingo diet (different from gulls) and feeding habitats could lead to greater exposure to this compound.

Flamingos are partial income breeders, meaning that the resources used for egg formation originate from overwintering areas and also from the breeding site (Rendón et al., 2011). From a contaminant transfer perspective, this reproductive strategy implies that part of the female contaminant burden transferred to the egg, and subsequently to the chick, may reflect the female exposure from both the wintering and the breeding grounds. As chicks' grow, the PFAS burden from the egg is diluted by the somatic growth (Sun et al., 2026). Blood is a highly dynamic matrix that reflects relatively recent exposure to contaminants. At two-months age, flamingo chicks are fed by a protein-rich crop milk produced by their parents (Ward et al., 2001). The transfer of contaminants from mothers to chick's through crop milk has not been studied yet. However, it has been hypothesized to be a relevant route of parental transfer of pollutants to their offspring (van Gessellen and Bouwman, 2025). Altogether, blood PFAS profiles at this stage are expected to predominantly reflect local environmental contamination rather than only the maternal transfer. Future studies should focus on the analysis of PFAS in eggs, crop milk, and adult diet to better disentangle the relative contribution of maternal transfer and dietary exposure. This research will identify which compounds are preferentially transferred from mother to egg versus those acquired directly through feeding, indicating potential local contamination.

3.2. Flamingo body condition

The body condition of birds is a predictive endpoint that mirrors the health status of the individuals (Peig and Green, 2010). Although it is a very general predictor, it can be used to evaluate changes in the health of the colony over time or in relation to different environmental stressors. The body condition in flamingo juveniles is a relevant factor in the migration behaviour of the species. Juveniles with better body condition are more prone to migrate than birds with lower body condition, which will rather stay at the natal area (Scridel et al., 2023). As a consequence, changes in juvenile flamingo body condition can have an impact in the population dynamics of this species in the Mediterranean metapopulation (Barbraud et al., 2003). In our study, flamingos' fledging's from 2022 to 2024 showed significantly better body condition ($p < 0.05$) compared to those from 2019.

Precipitation is known as a relevant environmental factor in flamingo fitness (Bechet and Johnson, 2008; Cezilly et al., 1995). Considering that, a linear mixed model was conducted with the SMI of individuals as response variable and the total precipitation during the breeding season (March–July), and the six PFAS compounds detected in more than 55% of the samples as explanatory variables (Table 2).

Among the three years, 2019 was the driest with a total precipitation from spring to mid-summer of 55.2 mm, whereas 2022 and 2024 had higher total rainfall of 214 mm and 117 mm, respectively. The results revealed a significant positive relationship between precipitation and fledgling body condition, suggesting that higher rainfall during the breeding season promotes better fledgling condition. These results are in accordance with observations in flamingos breeding in Camargue (South of France), where a positive correlation was found between the body condition of individuals and the water levels in the breeding area during the March-July period (Bechet and Johnson, 2008). The precipitation during the breeding season is important to flood temporary marshes, expanding the feeding areas. Yohannes et al. (2014) reported that flamingo chicks fed from temporary marshes were in better condition than those fed from permanent marshes in Camargue, due to a higher density of invertebrates in the temporary ones. A similar mechanism to that described in Camargue could potentially happen in the Ebro Delta. Flooded areas associated with higher precipitation are mainly located along coastal marshes and are not used by flamingos for feeding. Therefore, the improved body condition of fledglings observed in years with higher rainfall may instead be related to increased invertebrates' productivity in the main flamingos' feeding areas, driven by higher freshwater inputs and nutrient availability. This could enhance prey availability and improve foraging efficiency of adult flamingos, potentially allowing them to forage closer to the colony or feed the chicks more frequently. Reduced foraging effort combined with increased feeding rates may ultimately result in better chick body condition (Amat et al., 2007). Further studies linking precipitation patterns with prey availability and productivity in flamingo feeding habitats are needed to test this hypothesis.

Notably, even after accounting for precipitation, the model showed a negative relationship between PFHxS and PFHpA concentrations and body condition, indicating that fledglings with higher levels of these

Table 2
Summary of the Linear Mix-Model assessing the relationship between flamingo's body condition (SMI) and the individual PFAS detected above 55% of the samples and total precipitation for each year during the breeding season (March-July) at Ebro Delta. Bold values indicate statistically significant results.

Body Condition (SMI)			
Predictors	Estimate	Std. Error	t value
Intercept	2209	50.7	43.5
PFOS	38.5	37	1.04
PFOA	95.8	47.6	2.01
PFHxS	**−73.5**	**34.9**	**−2.11**
PFNA	−33.3	45.4	−0.73
PFHpA	**−61.6**	**29.2**	**−2.11**
PFDA	0.72	30.7	0.02
Precipitation	**207**	**56**	**3.69**

compounds tend to have poorer condition (Table 2). In contrast, the model showed a marginally positive association between PFOA concentrations and body condition. Although this positive correlation might appear contradictory, the higher PFOA levels in chicks in better condition could be a result of a more protein-rich diet rather than the effect of PFOA exposure itself. Flamingos with better body condition have been shown to present higher plasma protein levels, which are directly related to a protein-rich diet (Amat et al., 2007). Given that PFAS bind to plasma proteins, it is expected that those individuals with high plasma proteins levels would also present higher PFOA concentrations, as it is the most abundant compound detected. Despite differences in diet and ecological niche, similar patterns have been reported in other species exposed to PFAS, such as gulls where higher PFOS levels were linked to individuals in better body condition due to their protein- and lipid-rich diets (Lopez-Antia et al., 2021).

Regarding the environmental availability of these compounds, higher precipitation could theoretically lead to a dilution effect of contaminants in the feeding habitats, leading to lower exposure in flamingos. However, our results do not show a clear correlation between PFAS exposure and total rainfall. For instance, individuals from the driest year (2019) and the wetter year (2022) in our study presented similar PFAS concentrations (78.2 and 81.7 ng/mL respectively), suggesting that other factors may compensate for the potential dilution effect.

Several studies have reported significant relationships between the exposure to PFAS in birds and the disruption of thyroid hormones involved in energy metabolism (Ask et al., 2021; Sebastiano et al., 2021; Tartu et al., 2014). Birds exposed to high levels of PFAS and other contaminants may experience alterations in metabolic function, which can ultimately affect their body condition. For example, Sebastiano et al. (2023), reported a negative association between PFAS exposure and body condition in chicks from three *Larus species*, suggesting that the early-life exposure to PFAS may reduce the body condition of birds. Contrarily, Tartu et al. (2014) found a positive association between the body condition in male Black-legged Kittiwakes and higher levels of PFNA, highlighting that these relationships can vary depending on species, compound, and ecological context.

In flamingos, the association between PFAS and body condition was previously tested only in individuals from 2019, and no relationship was found (Dulsat-Masvidal et al., 2023). This lack of association may be explained by the relatively uniform exposure among individuals and the limited sample size ($n = 50$), which restricted statistical power. The results of the current study, however, suggest a potential negative relationship between PFHxS and PFHpA and the fledgling body condition (Table 2), indicating that larger datasets are needed to better understand this effect. Gillingham et al. (2021) found that flamingo chicks that bioaccumulated trace elements such as selenium (Se), mercury (Hg), and lead (Pb) exhibited lower body condition, supporting the idea that contaminant exposure may impair flamingo health. Overall, further research is needed to clarify the effects of PFAS and other organic contaminants on the body condition of flamingos.

3.3. Distribution and potential sources of PFAS in the Ebro Delta

Fifteen out of the 25 target PFAS were detected in all water samples ($n = 8$) across the EDNP, with $\sum$PFAS ranging from 22.9 to 65.8 ng/L. The detected compounds in water coincided with those in flamingos' blood, except for PFHpS, FOSA and PFTeDA which were found in flamingos but not in water samples. On the other hand, PFPeA, PFPeS and 6:2 FTS were detected in water but not in flamingos. The PFAS concentrations detected in each site are detailed in Table S4. Overall, PFAS were distributed across the study area, with the higher concentrations in locations affected by agricultural runoff (Fig. 3). The highest concentrations were measured at site 5 (65.8 ng/L), a constructed wetland that filters agricultural runoff, and at site 3 (45.4 ng/L) and 1 (38.9 ng/L), which are agricultural channels receiving water from rice fields (Fig. 3).

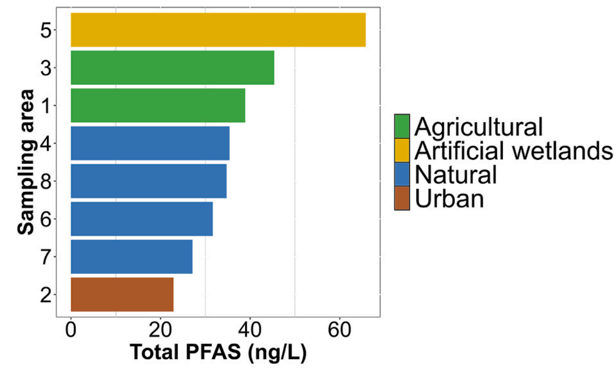

Fig. 3. Total concentrations of PFAS (ng/L) detected in the water samples. Colours indicate the main land use.

PFCA were more prevalent than PFSA in water samples (Table S4). Short-chain PFCA (C4–C6) were detected in all samples and at the highest concentrations, with PFHxA ranging from 7.47 ng/L at site 2 (urban area) to 18.7 ng/L at site 5 (artificial wetland), followed by PFBA (2.48 to 13.3 ng/L) and PFPeA (0.75 to 10.6 ng/L). Among short-chain PFSAs, PFBS was detected in all samples (0.49–7.14 ng/L) and PFHxS in all except site 7 (natural site), ranging from 0.48 to 3.19 ng/L. Long-chain PFCA were also present at relevant levels. PFUdA was detected in seven samples (3.64–13.9 ng/L), PFOA was found in all samples (1.15–4.08 ng/L), PFNA was also present in all sites but at lower concentrations (0.65 to 1.12 ng/L). PFOS (long-chain PFSA) was found in 6 sites at low concentrations, from 0.25 to 0.95 ng/L. Pignotti et al. (2017) also reported PFOA as a predominant compound in water and sediment from the Ebro Delta at higher levels than PFOS. The study also reported the final part of the Ebro river, including *L'Encanyissada* (site 6), as one of the most affected sites by PFAS since it receives intermittent inputs of drainage water from rice fields, particularly when irrigation water is not available.

The PFAS contamination profile in water exhibited a distinct fingerprint compared to that detected in blood samples (Fig. S1). Water samples were characterized by a higher contribution of short-chain PFAS (PFBA, PFHxA and PFBS), whereas the fingerprint of flamingo chicks was dominated by long-chain compounds more prone to bioaccumulation (PFOA, PFOS and PFNA) (Fig. S1). This divergence is explained by the fact that flamingo chicks are not directly exposed to water but are instead fed with crop milk by their parents. Furthermore, short-chain PFAS are more readily metabolized and excreted by biota compared to the more persistent long-chain compounds prevalent in blood. Consequently, a direct relationship of pollution fingerprints between blood and water is not expected. However, when focusing on the PFAS bioaccumulated in chicks (those detected in >55% of flamingos), significant correlations ($p < 0.05$) were found with PFAS patterns water from agricultural areas (sites 1 and 3), the urban sampling point (site 2), natural sites (4 and 6), and the agricultural wetland (site 5) (Fig. S2). These correlations support the hypothesis that local contamination in the Ebro Delta represents a PFAS source to the colony. Future studies incorporating the analysis of adult diet and crop milk will be essential for more definitive source apportionment.

Agriculture is the main economic activity in the Ebro Delta, in particular rice cultivation, which accounts for approximately 62% of the total cropland (Fatorić and Chelleri, 2012). Rice fields, besides their economic importance, provide a valuable habitat for waterbirds including flamingos (Paulino et al., 2025). The abundance of flamingos feeding in rice fields has been even identified as a problem for rice production (Tourenq et al., 1999), which is indicative of the extensively use of this resource for the species. Except for site 2 (urban), which represents the main river inflow (Fig. 2), all sampling sites included in our study are located within the Natural Park, suggesting that adult flamingos may be exposed to PFAS while foraging in these sites or surrounding areas.

The widespread detection of PFAS in our study is likely due to multiple sources of PFAS pollution, including agricultural practices. PFAS can reach agricultural fields through the application of pesticide formulations, irrigation with river water affected by waste water treatment plant discharges, application of biosolids or municipal composts, and less likely atmospheric deposition (Costello and Lee, 2024). Although there is still little research on the subject, PFAS and their degradation products were reported in pesticide formulations from the United States due to their use as active ingredients or coadjutants, and at some extent due to leaching from fluorinated containers (Donley et al., 2024). This emerging contamination pathway challenges conventional assumptions about PFAS sources into the agricultural environment. Pesticides with fluorinated moieties (-CF_3) within their structure are known as PFAS pesticides. According to the Non-Governmental Organization *Pesticide Action Newtwork*, 37 pesticides approved for use in Europe contain -CF_3 groups in their structure, making them potential PFAS precursors (PAN, 2023). Two of these, Penoxsulam (Herbicide) and Flonicamid (Insecticide) are approved for use in rice fields in Spain (Ministry of Agriculture Fisheries and Food, 2025). There is a growing environmental concern regarding the use of these products, and recently Denmark has withdrawn pesticides that contain fluorinated groups in their structure as can form trifluoracetic acid (TFA), an ultra-short chain PFAS, but also other PFAS (Danish Environmental Protection Agency, 2025). A recent study across three important economical and agricultural regions (Europe, the United States of America and China) stated that TFA formation from pesticides and plant protection products (PPP) is estimated at maximum emissions ranging from 0 to 83 kg/km^2 per year (Joerss et al., 2024). To our knowledge, the role of pesticides as PFAS precursors in agriculture has not yet been studied in Spain. Future monitoring programmes in the Ebro Delta will unveil whether PFAS-containing pesticides used in rice fields represent a potential source of PFAS in the area.

4. Conclusion

The Ebro Delta breeding colony of flamingos is chronically exposed to PFAS, as all analysed chicks contained relevant concentrations of PFOA, PFOS and PFHxS. The decline in PFOA and PFHxS concentrations over the five-year period is consistent with recent regulatory phase-out of these compounds in Europe, highlighting the value of long-term biomonitoring studies for assessing the effectiveness of chemical regulations.

Chicks body condition improved in wetter years during the breeding season (2022 and 2024), likely due to an enhanced food availability. Nevertheless, the body condition was negatively related to higher concentrations of PFHxS and PFHpA, suggesting potential adverse effects of PFAS exposure during early development. The widespread detection of PFAS in waters from the Natural Park supports local contamination as a source of PFAS exposure. Further research is needed to understand the PFAS exposure pathways to the colony.

The results underscore the need to integrate contaminant monitoring programmes into conservation and management strategies in the Ebro Delta Natural Park to support management actions aimed at reducing PFAS inputs as limiting the discharge of agricultural runoff into sensitive habitats used by birds during the breeding season.

CRediT authorship contribution statement

Maria Dulsat-Masvidal: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing. **Bernat Oró-Nolla:** Formal analysis, Methodology, Writing – review & editing. **Albert Bertolero:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Sílvia Lacorte:** Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Validation, Writing – review & editing.

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

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