



# How does habitat use influence PFAS contamination in wildlife? Combining stable isotopes and GPS tracking in three gull species<sup>☆</sup>

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## ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are toxic and persistent compounds widely distributed in the environment and accumulate in top predators, including seabirds. Because of the biomagnification potentials of some PFAS, diet is thought to be a key exposure route for PFAS. However, other factors such as habitat use, may mask interspecific differences in PFAS exposure expected from trophic structure. Among seabirds, gulls are generalists that forage in both terrestrial and marine habitats, making them relevant models to concurrently investigate the influence of foraging habitats and trophic position on PFAS exposure. We combined plasma PFAS concentrations with GPS tracking and stable isotopes to define foraging habitats ( $\delta^{13}\text{C}$ ;  $\delta^{34}\text{S}$ ; GPS) and trophic positions ( $\delta^{15}\text{N}$ ) in three sympatric gull species breeding in France (Isle of Ré). In herring gulls (*Larus argentatus*), long-chain perfluoroalkyl carboxylic acids (PFCAs) were positively correlated with high trophic resources from marine habitats. We found compound- and sex-dependent relationships between PFAS concentrations and stable isotope values in lesser black-backed gulls (*Larus fuscus*), while no association was found with habitat use. No association was found between PFAS levels and stable isotopes in great black-backed gulls (*Larus marinus*). Our study suggests that coastal habitat could be a source of PFCA contamination and highlights that the influence of habitat use on gull exposure to PFAS varied depending on species, sex, and compounds.

## 1. Introduction

Per- and poly-fluoroalkyl substances (hereafter PFAS) include thousands of synthetic organic compounds (Schymanski et al., 2023) which are widely used in many manufacturing products (e.g., cosmetics, food

packaging, non-stick cookware, waterproof textiles, medical devices, pesticides, and firefighting foams) (Gaines, 2023). With a stable chemical structure formed by fluorine atoms attached to a carbon chain (Buck et al., 2011), PFAS have unique chemical and physical properties that make them extremely persistent in the environment ("Forever

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chemicals"). PFAS are now ubiquitous and commonly found in humans and wildlife tissues worldwide (De Silva et al., 2021; Sinclair et al., 2020). This led to growing concerns about the consequences of PFAS exposure for human health and the environment (DeWitt, 2015; Fenton et al., 2021). Indeed, PFAS are reported to be carcinogenetic, immunotoxic, neurotoxic, and to act as endocrine-disruptors (Fenton et al., 2021; Ma et al., 2023, 2022). Consequently, three PFAS and their salts (perfluorooctanesulfonic acid -PFOS-, perfluorooctanoic acid -PFOA- and perfluorohexanesulphonic acid -PFHxS-) have been listed under the Stockholm Convention on Persistent Organic Pollutants (United Nations, 2020).

In wildlife, seabirds are commonly used as bioindicators of environmental pollution (Elliott and Elliott, 2013), because they accumulate significant levels of PFAS with detrimental effects on their physiology and fitness (e.g., Ask et al., 2021; Blévin et al., 2017a, 2017b; Costantini et al., 2022; Humann-Guillemot et al., 2024; Melnes et al., 2017; Sebastiano et al., 2023, 2020; Tartu et al., 2014). Due to the biomagnification potentials of some PFAS (Conder et al., 2008; Lewis et al., 2022), concentrations are significantly higher in seabirds situated at the top of marine food webs suggesting that diet represents a key exposure route for PFAS (Alfaro Garcia et al., 2022; Borgå et al., 2004; Chételat et al., 2020; Roscales et al., 2019). To assess the trophic ecology of seabirds, stable isotope analyses are commonly used with high carbon and sulphur values ( $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$ , respectively) as indicators of more marine feeding habitats and diet, and higher nitrogen values ( $\delta^{15}\text{N}$ ) as indicator of higher trophic position (Forero and Hobson, 2003; Hobson et al., 1994). Some studies have reported positive associations between  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values and PFAS concentrations in diverse matrices of seabirds (e.g., eggs, feathers, liver, muscle, or plasma) (Gebbink et al., 2011; Padilha et al., 2022; Robuck et al., 2020). However, other studies reported conversely negative or no correlations between stable isotope values and PFAS concentrations (Bustnes et al., 2013; Carravieri et al., 2020; Gebbink et al., 2011; Hebert et al., 2022; Leat et al., 2013; Padilha et al., 2024; Robuck et al., 2020), suggesting that other factors, such as spatial movements, and habitat use may however mask interspecies differences in PFAS expected from trophic structure alone (Roscales et al., 2019). For instance, it has been suggested that the different marine environments (coastal or offshore) can influence PFAS exposure (Gebbink et al., 2011; Miller et al., 2015).

Biologging (e.g., using GPS, satellite or geolocator (GLS) devices) can provide unparalleled fine-scale information on the spatial movements of seabirds (Burger and Shaffer, 2008). The use of such loggers in addition to stable isotope values can be of great interest for understanding the origin of seabird contamination (Baak et al., 2024; Elliott and Elliott, 2013). However, studies linking contaminant loads to avian movements have mainly focused on other substances (e.g., trace elements or polybrominated diphenyl ethers) than PFAS, which have to our knowledge received little attention (reviewed in Baak et al., 2024). Previous work using GLS have investigated the influence of the wintering areas on blood PFAS concentrations of black-legged kittiwakes (*Rissa tridactyla*) and ancient murrelets (*Synthliboramphus antiquus*) (Léandri-Breton et al., 2024; Miller et al., 2020), and suggests that migratory seabirds can act as biovectors of PFAS to Arctic nesting sites (Léandri-Breton et al., 2024). Regarding GPS, a limited number of studies have used this technology to investigate the link between PFAS contamination and fine-scale foraging movements during the breeding season (but see Jang et al., 2022; Wilkinson et al., 2022). However, it is essential to use the fine spatial resolution of PFAS contamination to identify habitats that are particularly exposed to PFAS and therefore species that are potentially vulnerable to PFAS contamination.

In the present study, we investigated the associations between plasma concentration of PFAS and habitat use, with two complementary approaches: stable isotopes ( $\delta^{13}\text{C}$ ,  $\delta^{34}\text{S}$ , and  $\delta^{15}\text{N}$ ) and fine-scale GPS tracking. We focused on three species of gulls (European herring gulls, herring gulls, hereafter, *Larus argentatus argenteus*, great black-backed gulls, *Larus marinus*, and lesser black-backed gulls, *Larus fuscus*

*graellsii*) breeding in sympatry in a Natural Reserve in the south-west France. On this site, these three species showed high plasma PFAS concentrations with interspecies variations (Sebastiano et al., 2023, 2021), but for which contamination sources are not identified. These species are opportunist and generalist feeders, being scavengers, and predators, collecting marine food at the sea surface, human wastes in landfills, and earthworms in fields (Camphuysen et al., 2015; Gyimesi et al., 2016; Steenweg et al., 2011; Pennycott et al., 2020). Used habitats include offshore, coastal, and terrestrial environments with inter-individual variation depending on the species (Jouanneau et al., 2022; O'Hanlon et al., 2025). Herring gulls use primarily terrestrial/coastal habitats while great black-backed gulls and lesser black-backed gulls use mainly coastal habitats or a mixture of terrestrial and offshore habitats, respectively (Jouanneau et al., 2022; O'Hanlon et al., 2025). This multi-species sampling allows to assess if foraging ecology, proxied by stable isotopes and localization of foraging sites with GPS-tracks, is an important driver of PFAS concentrations for these three species and between individuals. We hypothesized that i) high trophic resources ( $\delta^{15}\text{N}$ ) from marine foraging habitats ( $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$ , or revealed by GPS-tracks) would be associated to higher PFAS concentrations, as marine resources are often reported as a potential contamination pathway for seabirds (Cheng et al., 2025; Colomer-Vidal et al., 2022). We also expected that associations between PFAS concentrations and foraging ecology could be ii) dependent of the chemical structure of the PFAS, bioaccumulation and biomagnification potentials being related to increasing fluorinated carbon chain, especially in perfluorosulfonic acids (PFSAs, hereafter) and the head group, as PFSAs have higher potentials than perfluorocarboxylic acids (PFCAs, hereafter) for a same chain length (Conder et al., 2008; Lewis et al., 2022); and iii) sex-specific, as we are aware of sex differences in foraging habitats, especially for lesser black-backed gulls (Camphuysen et al., 2015).

## 2. Material and methods

PFAS concentrations and movement data used in the present study have been independently described in two previous publications: PFAS concentrations were described in Sebastiano et al. (2021), while isotopic and movement analyses were discussed in Jouanneau et al. (2022). In the present study, we focus on the association between PFAS concentrations and the foraging strategies of the species.

### 2.1. Study site and species sampling

Fieldwork was conducted during the breeding period in May 2016, 2017, and 2018 in the "Lilleau des Niges" Natural Reserve, Isle of Ré, France (46°13'53"N, 1°30'22"W). Lilleau des Niges is a 1.21 km<sup>2</sup> protected area located in the west part of the continental island, composed of salt marshes and mudflats managed by the Ligue de Protection des Oiseaux (LPO). The sampled site is located at ~30–50 km to three river mouths (the Sèvre Niortaise, the Charente, and the Seudre) where PFAS have been previously detected in mussels (Munsch et al., 2019, 2013; Serre et al., 2025). To date, no fluorochemical plant or PFAS-impacted industrial sites are known in this area (>20 km, Cordner et al., 2024; Ministry of Ecological Transition, Biodiversity, Forests, Sea, and Fisheries, 2025) and the nearest airport is located >20 km. This natural reserve hosts several breeding species including the three focal species: the herring gull, the lesser black-backed gull, and the great black-backed gull.

We sampled 108 adults from the three species (35 herring gulls, 44 lesser black-backed gulls, 29 great black-backed gulls, details on sample sizes per species, year and sex are summarized in Table S1). Three individuals were excluded from further physiological analyses because we were unable to perform morphological measurements or blood sampling (two of them were included in movement tracking analyses, see below). Two individuals have been sampled twice in different years; we thus randomly chose one observation in the next analyses to avoid pseudo-

replication.

All sampled gulls were incubating eggs and were captured with a trap installed on the nest as previously described (Jouanneau et al., 2022). Birds were ringed with both metal and plastic leg rings engraved with a unique alphanumeric combination if it had not been ringed previously. After morphological and body mass measurements (Sebastiano et al., 2021), we collected 2 mL of blood from the brachial vein. Upon return to the laboratory, blood was centrifuged at 8,000 rpm for 10 min to separate the plasma and red blood cells. All samples were then stored at  $-20^{\circ}\text{C}$ .

## 2.2. Individual foraging behaviour

A subset of individuals ( $n = 43$  in total, including the two individuals that were not blood sampled) was also equipped with GPS-UHF loggers (Harrier Ecotone®, see Table S1 for details on sample sizes by species, year, and sex). The total mass of the logger and the attachment (harness) was on average 13 g, i.e., 2 % or less of the weight of the individuals. We recorded locations of the bird every 5 min from the day following blood sampling until either hatching or nest failure. Period of raw data recording ranged from four days (two individuals) to 41 days (one individual) with an average of  $16 \pm 8$  (SD) days (Table S2). Despite the fact that tracking took place after blood sampling, we considered the behaviours extracted from these locations to be representative of the foraging trips that could explain blood PFAS concentrations, since these gulls have previously been shown to exhibit high levels of individual foraging site fidelity (Jouanneau et al., 2022).

We identified foraging behaviour in GPS tracks by classifying each location as one of two behavioural states (commuting vs. resting/foraging) using Hidden Markov models (Franke et al., 2004) with the moveHMM package in R (Michélot et al., 2016). A foraging site was then defined as the centroid of consecutive resting/foraging locations during a foraging trip. We identified whether the site was in a marine (offshore or coastal: including salt marshes and intertidal zones) or in a terrestrial habitat (any other) based on the CORINE Land cover dataset (CORINE Land Cover, Feranec et al., 2016). For each individual, we calculated the proportion of the foraging sites located in a marine environment, with the hypothesis that this indicates a more marine diet, hereafter called the “marine foraging index”. The complete procedure of the foraging sites identification is described in Supplementary Information 1.

## 2.3. Molecular sexing, stable isotopes, and PFAS

All individuals were sexed at the Centre d'Etudes Biologiques de Chizé (CEBC) using red blood cell samples by polymerase chain reaction amplification of two highly conserved genes of the sex chromosomes (Fridolfsson and Ellegren, 1999).

Stable isotope analyses were conducted at Littoral Environnement et Sociétés (LIENSs). The relative abundance of the three isotopes were measured from weighted sub-sample of red blood cells with a flow mass spectrometer (Thermo Scientific Delta V Advantage) coupled with an elemental analyzer (Thermo Scientific Flash EA for carbon and nitrogen; Thermo Scientific Flash EA IsoLink for sulphur). Isotope values were calculated following:  $\delta^{13}\text{C}$ ,  $\delta^{34}\text{S}$ , and  $\delta^{15}\text{N} = ((R_{\text{sample}}/R_{\text{standard}}) - 1) \times 1000$  with  $R$  being the ratio  $^{13}\text{C}/^{12}\text{C}$ ,  $^{34}\text{S}/^{32}\text{S}$ , or  $^{15}\text{N}/^{14}\text{N}$ , respectively. Using internal laboratory standard, all analytical precisions were  $<0.2\%$ . Isotopic values were presented using the standard delta ( $\delta$ ) notations based on international standards, Vienna Pee Dee Belemnite, Vienna Cañon Diablo troilite and atmospheric nitrogen ( $\text{N}_2$ ) for  $\delta^{13}\text{C}$ ,  $\delta^{14}\text{S}$ , and  $\delta^{15}\text{N}$ , respectively.

Eleven different PFAS were quantified in plasma samples as described in Sebastiano et al. (2021): three perfluoroalkylsulfonic acids – PFOS (linear and branched form: L-PFOS and Br-PFOS, respectively), PFHpS (L-PFHpS and Br-PFHpS), PFHxS –, seven PFCAs – PFOA (L-PFOA and Br-PFOA), PFDA, PFNA, PFUnDA, PFDODA, PFTeDA, and PFTTrDA –, one perfluoroalkane sulfonamide – FOSA –. Chemical names and

structures are provided in Table S3. Only PFAS detected in  $>70\%$  of samples were statistically analysed (i.e., all PFAS, except FOSA, PFOA (L-PFOA and Br-PFOA), and Br-PFHpS). Detection frequencies, limit of detections and quantification are defined and provided in Sebastiano et al. (2021, Table S1).

## 2.4. Statistical analyses

All analyses were performed with R version 4.2.1 “Funny-Looking Kid” (R Core Team, 2020). As in Jouanneau et al. (2022), a principal component analyses (PCA with “FactoMineR” R package, Lê et al., 2008) was performed to reduce the number of explanatory variables and included the three stable isotopes, highly and positively correlated to each other (see Figs. S1 and S2 and Pearson correlations  $p < 0.001$  for all). The two first dimensions of the PCA ( $\text{PC}_1$  and  $\text{PC}_2$ , hereafter) were kept and explained 95 % of the total variance.  $\text{PC}_1$  was influenced by all three isotope ratios:  $\delta^{13}\text{C}$  values (factor loading: 0.93),  $\delta^{15}\text{N}$  values (0.93), and  $\delta^{34}\text{S}$  values (0.89). The  $\text{PC}_2$  was only significantly influenced by  $\delta^{34}\text{S}$  values (0.47). For both  $\text{PC}_1$  and  $\text{PC}_2$ , high scores indicated high  $\delta^{13}\text{C}$ ,  $\delta^{34}\text{S}$ , and  $\delta^{15}\text{N}$  values. Individual scores for each PC were extracted to use them as predictors in statistical models.

A second PCA was used to examine correlations between PFAS. Most PFAS, whether PFSAs or PFCAs were highly positively correlated with each other (except PFHxS), as revealed by the PCA (Fig. S3). All PFAS were thus included as response variables in multivariate regression models to explore compound-specific associations with predictors taking account for multiple dependences into PFAS.

First, we aimed to investigate the influence of stable isotopes (using  $\text{PC}_1$  and  $\text{PC}_2$ ), species, and sex of all individuals ( $n = 105$ ) on plasma PFAS concentrations using multivariate multiple regression. First, for each PFAS independently, concentrations were log-transformed to approach a Gaussian distribution. The initial linear model included PFAS concentrations as the response variable, and  $\text{PC}_1$ ,  $\text{PC}_2$ , their respective interactions with species or sex, and year as additive explanatory variable. We checked multicollinearity of explanatory variables for each model using variance inflation factors (Zuur et al., 2010). Large variance inflation factor scores ( $\text{GVIF}^{1/(2.\text{df})} > 2$  or  $\text{VIF} > 4$ ) indicated high multicollinearity between species and PCs variables and their interactions, precluded us from running models combining species (see Table S4). Similarly, large variance inflation factor scores precluded us from running models combining sexes in lesser black-backed gull and great black-backed gull models while there was no indication of multicollinearity in the herring gull model (see Table S5). The best models were backward selected based on Pillai's trace tests. Year was however not included in models in the case great black-backed gulls as sample size was too low (denominator degree of freedom in Pillai's test = 0). The importance of outliers was assessed by refitting all models without data showing a Cook's distance higher than 0.5 but the inclusion of such outliers did not qualitatively change the results of the analyses, and they were therefore kept in the rest of the manuscript.

Secondly, we analysed the relationship between the marine foraging index and log-transformed PFAS concentrations in birds for which we had both PFAS and GPS data for each species and sex (17 herring gulls: 9 females/8 males; 18 lesser black-backed gulls: 8/10). Great black-backed gulls were excluded from spatial analyses due to limited sample size ( $n = 6$ , 3 females/3 males) which would impact statistical power. PFAS concentrations were used as response variables in a multivariate regression model with the marine foraging index and its interaction with the sex and the species, as well as the year as additive explanatory variables. We also identified multicollinearity of the marine foraging index with the species (Table S6) and sex (Table S7) variables precluding us from running models combining these variables. Sex separation for multicollinearity purposes with the two-interaction of the marine foraging index with sex reduced drastically the statistical power owing to our modest sample sizes; therefore, both sexes were analysed in the same model with sex as an additive effect (no multicollinearity in

this case). We assessed the robustness of our results to the removal of observations in Supplementary Information 3.

For all the best models, heteroscedasticity and normality of residuals were checked and a significance level of  $\alpha < 0.05$  was used for model selection procedures (when removing a variable with a marginal effect, results with and without the variable are provided). The model selection results, and the variance explained by each parameter of the best model are described in Table S8–S11. Predicted coefficients ( $\beta$ ) presented in the results are shown with their standard errors.

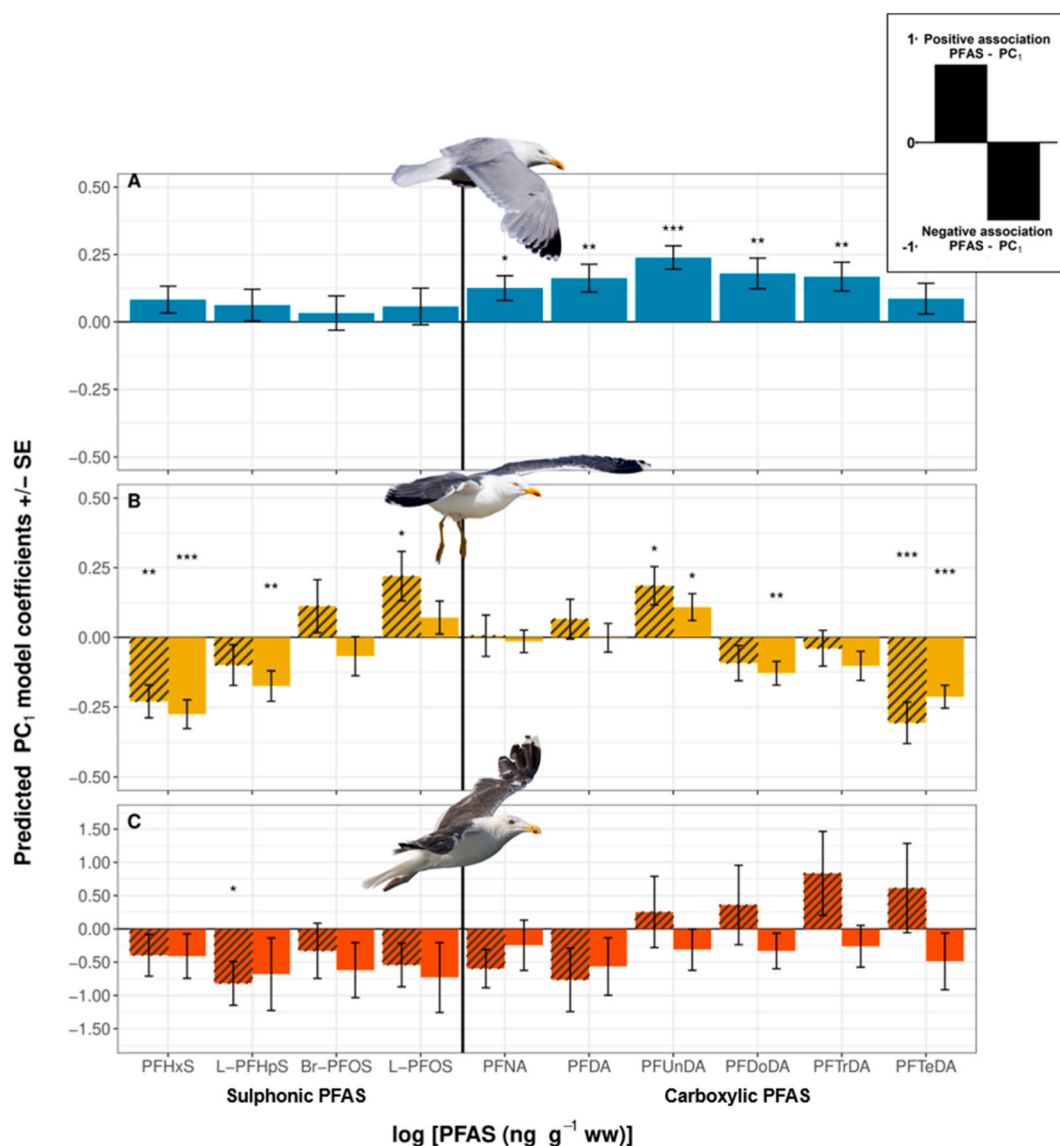
### 3. Results

#### 3.1. Associations between foraging ecology and PFAS concentrations using stable isotopes

In herring gulls, PC<sub>1</sub> significantly explained inter-individual

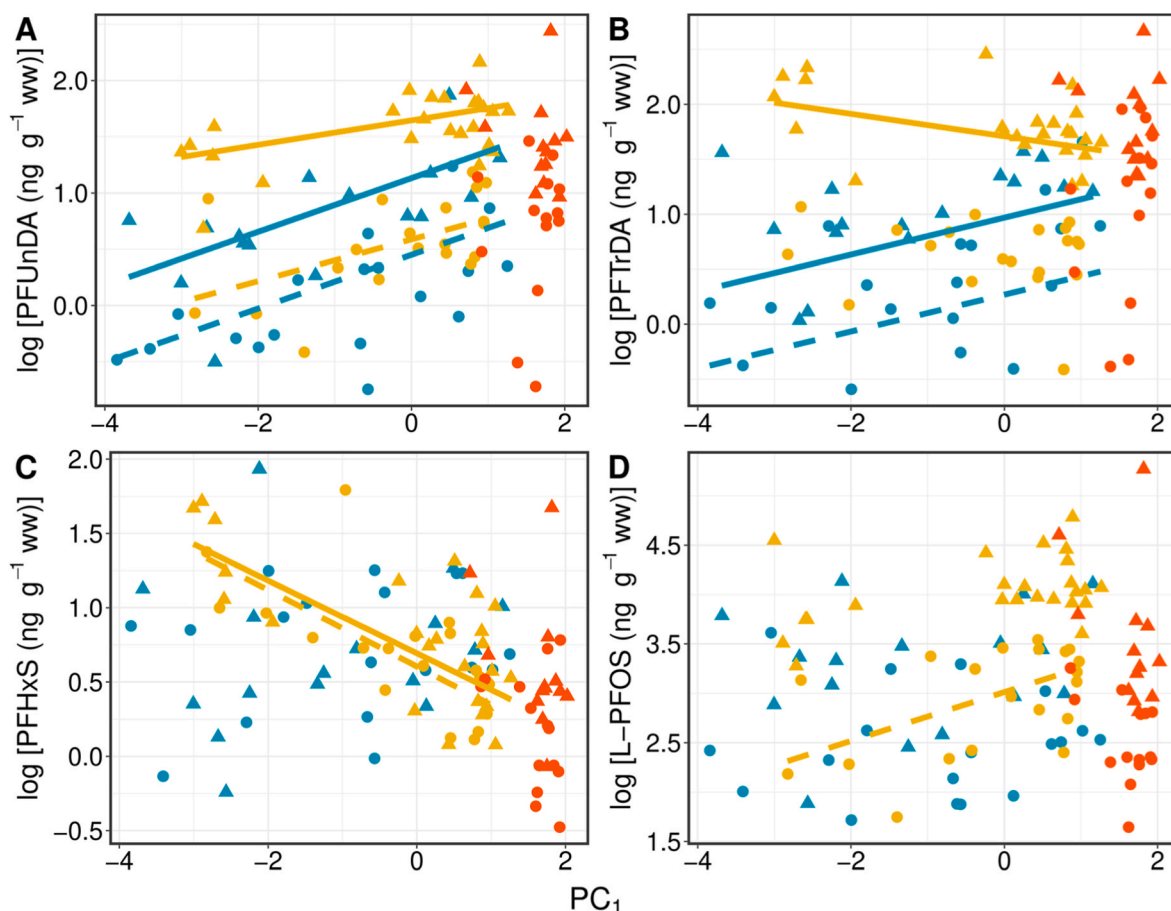
variations in blood PFAS concentrations (Table S8;  $F_{10,20} = 4.92$ ,  $p = 0.001$ ). Blood concentrations of PFCAs (excluding PFTeDA) were significantly and positively associated with PC<sub>1</sub> (all  $p < 0.05$ ; all  $\beta > 0.1$  excluding  $\beta_{\text{PFTeDA}} = 0.09 \pm 0.06$ ,  $t = 1.52$ ,  $p = 0.14$ ; Fig. 1A and 2A and 2B), whereas no significant relationships were found between PFASs and PC<sub>1</sub> (Fig. 2C and 2D). With the exception of PFHxS, all PFAS concentrations were higher in males than in females ( $F_{10,20} = 5.6$ ,  $p < 0.001$ ; all  $\beta \geq 0.37$  and all  $p \leq 0.02$ , excluding  $\beta_{\text{PFHxS}} = -0.10 \pm 0.05$ ,  $t = -0.6$ ,  $p = 0.5$ , Fig. 2).

In lesser black-backed gulls, PC<sub>1</sub> also explained the inter-individual variations in blood PFAS concentrations in both the female-only and the male-only models (females:  $F_{10,9} = 36.0$ ,  $p < 0.001$ ; males:  $F_{10,131} = 10.2$ ,  $p < 0.001$ ; Table S9). In females of lesser black-backed gulls, L-PFOS and PFUnDA concentrations were positively correlated with PC<sub>1</sub> (Fig. 1B; Fig. 2D:  $\beta_{\text{L-PFOS}} = 0.22 \pm 0.09$ ,  $t = 2.5$ ,  $p = 0.02$ ; Fig. 2A:  $\beta_{\text{PFUnDA}} = 0.19 \pm 0.07$ ,  $t = 2.7$ ,  $p = 0.01$ ), whereas PFHxS and PFTeDA



**Fig. 1.** Direction and significance of PC<sub>1</sub> scores in multivariate linear regressions to PFAS concentrations. A: herring gull (blue); B: lesser black-backed gull (yellow); C: great black-backed gull (red). PFAS are classified by chemical structure (sulfonic and carboxylic) and ordered by carbon chain length. The height of each bar represents  $\beta_X$  of PC<sub>1</sub> (predicted linear coefficient) for PFAS X. If  $\beta_X > 0$  (resp.  $< 0$ ), then the concentration of PFAS X increases (resp. decreases) when PC<sub>1</sub> increases (resp. decreases) (see small panel). Error bars are standard errors. The stars represent the significance of the  $t$ -test comparing the difference of  $\beta_X$  to 0: \*:  $0.05 \geq p > 0.01$ ; \*\*:  $0.01 \geq p > 0.001$ ; \*\*\*:  $0.001 \geq p$ , and their absence means no significance. Hatched and plain bars are for females and males, respectively. Note that the best model explaining variations in the PFAS concentrations in males great black-backed gulls is the null model. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)





**Fig. 2.** Relationships between PFAS concentrations and PC<sub>1</sub> related to stable isotopes values, with four representative PFAS (two carboxylic PFAS and two sulfonic PFAS) chosen as an example. A: PFUnDA; B: PFTTrDA; C: PFHxS and D: L-PFOS as a function of PC<sub>1</sub>. Higher PC<sub>1</sub> scores are related to higher stable isotopes values (carbon, sulphur, and nitrogen). Each point represents an individual. Lines are predicted significant linear relationships between PFAS concentrations and PC<sub>1</sub> averaged per year. Colours indicate the species with European herring gull, lesser black-backed gull and great black-backed gull in blue, yellow, and red, respectively. Line types and shape points are different for females and males, with dashed lines and circles for females and plain lines and triangles for males. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

concentrations were negatively correlated with PC<sub>1</sub> (Fig. 2C:  $\beta_{PFHxS} = -0.23 \pm 0.06$ ,  $t = -3.9$ ,  $p = 0.001$ ;  $\beta_{PFTTrDA} = -0.31 \pm 0.07$ ,  $t = -4.1$ ,  $p < 0.001$ ). All other PFAS concentrations were not correlated with PC<sub>1</sub> (all  $p > 0.16$ ). For male lesser black-backed gulls, PFUnDA concentrations were positively correlated with PC<sub>1</sub> (Fig. 1B and 2A: PFUnDA:  $\beta_{PFUnDA} = 0.11 \pm 0.055$ ,  $t = 2.3$ ,  $p = 0.04$ ), while PFHxS, L-PFHps, PFDoDA, and PFTTrDA concentrations were negatively correlated with PC<sub>1</sub> (Fig. 1; Fig. 2B and C: all  $\beta \leq -0.13$  and all  $p < 0.007$ ). Concentrations of all the other PFAS were not correlated to PC<sub>1</sub> (all  $p > 0.05$ ). In great black-backed gulls, PC<sub>1</sub> significantly explained the inter-individual variations in concentrations of PFAS in females only ( $F_{10,3} = 28.8$ ,  $p = 0.01$ ; Table S10), while the best model for males was the null model. For females, only concentrations of L-PFHps were significantly and negatively correlated with PC<sub>1</sub> ( $\beta_{L-PFHps} = -0.82 \pm 0.33$ ,  $t = -2.51$ ,  $p = 0.03$ , Fig. 1C), while all the other PFAS were not correlated (all  $p > 0.05$ ).

For all species and models, PC<sub>2</sub> was never retained in the best models, indicating that PC<sub>2</sub> did not significantly explain variation in PFAS concentrations (Table S8–S10).

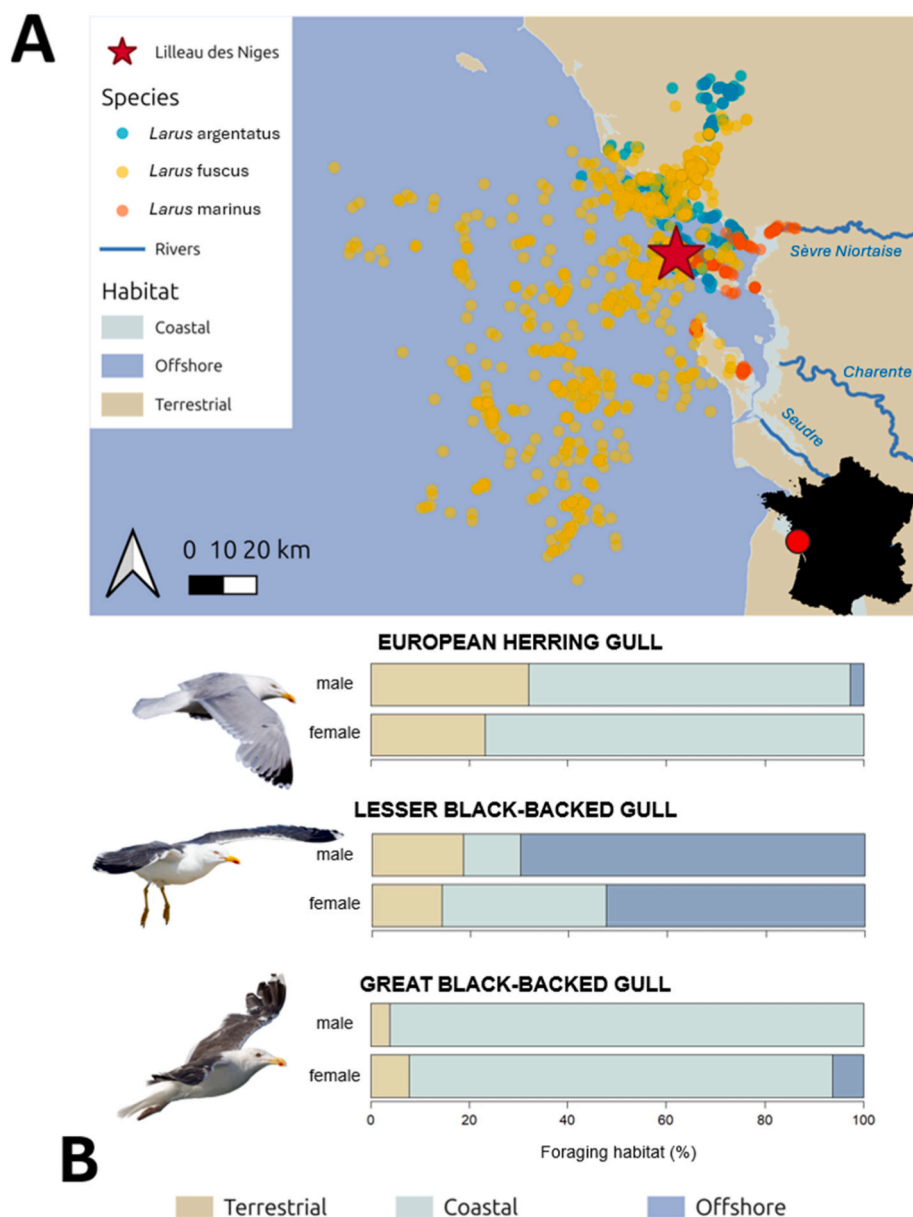
### 3.2. Associations between foraging ecology and PFAS concentrations using GPS tracking

GPS tracks revealed that habitat use was species-dependent: herring gulls and lesser black-backed gulls showed a high inter-individual variability, foraging in diverse habitats from terrestrial to offshore. Both herring gulls and lesser black-backed gulls used terrestrial habitats,

but herring gulls were mostly related to urban or even landfill areas, while lesser black-backed gulls mostly forage in fields. Lesser black-backed gulls also used offshore habitats, up to 100 km from the colony (Fig. 3A), likely foraging on fisheries discards while herring gulls restricted their use of marine environments to coastal habitats. Compared to the two other species, great black-backed gulls foraged almost exclusively in coastal habitats, including river mouths, with low individual variability (Fig. 3A).

In herring gulls, the marine foraging index significantly explained inter-individual variations in PFAS concentrations (Table S11 and Fig. 4A;  $F_{10,4} = 8.82$ ,  $p = 0.03$ ) while sex had a marginal non-significant effect ( $F_{10,5} = 4.81$ ,  $p = 0.07$ ). All concentrations of PFCAs (except PFTTrDA) as well as the PFASs were positively correlated with the marine foraging index (all  $\beta > 0.88$ , all  $p \leq 0.04$ ; Fig. 4A and 5). However, when the marginal effect of sex was removed during model selection ( $p = 0.07$ ), the effect of the marine foraging index on PFAS concentrations was only marginal ( $F_{10,5} = 3.25$ ,  $p = 0.1$ ). In this case, only PFNA, PFDA, PFDoDA, PFUnDA, and PFHxS concentrations were significantly associated with the marine foraging index ( $\beta_{PFNA} = 1.00 \pm 0.38$ ,  $t = 2.63$ ,  $p = 0.02$ ;  $\beta_{PFDA} = 1.45 \pm 0.50$ ,  $t = 2.91$ ,  $p = 0.01$ ;  $\beta_{PFUnDA} = 1.45 \pm 0.54$ ,  $t = 2.68$ ,  $p = 0.02$ ;  $\beta_{PFDoDA} = 1.30 \pm 0.54$ ,  $t = 2.42$ ,  $p = 0.03$ ; Fig. 5C:  $\beta_{PFHxS} = 0.89 \pm 0.34$ ,  $t = 2.59$ ,  $p = 0.02$ ), and all other PFAS concentrations were unrelated to the marine foraging index.

In lesser black-backed gulls, the marine foraging index did not explain the differences in PFAS concentrations between individuals (Table S11 and Fig. 4B). The best model included only sex ( $F_{10,7} = 28.98$ ,



**Fig. 3.** A. Visualisation of the identified foraging sites for each species around the colony “Lilleau des Niges” in the Isle of Ré. Each point indicates a foraging site in the map. Each species is indicated by a colour with European herring gull, lesser black-backed gull, and great black-backed gull in blue, yellow, and red, respectively. Terrestrial, coastal, and offshore habitats are indicated in brown, light blue, and dark blue, respectively. B. Percent stacked barplots of the proportion of foraging sites in different foraging habitats for each sex of the three species: the European herring gull ( $n = 17$ , 8 males/9 females), the lesser black-backed gull ( $n = 18$ , 10 males/8 females) and the great black-backed gull ( $n = 6$ , 3 males/3 females). Percent in the stacked plots correspond to median proportions. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

$p < 0.001$ ) with males having higher concentrations than females for all PFAS (all  $\beta > 0.68$ , all  $p < 0.001$ ), except for PFHxS ( $\beta_{PFHxS} = 0.33 \pm 0.22$ ,  $t = 1.50$ ,  $p = 0.15$ ).

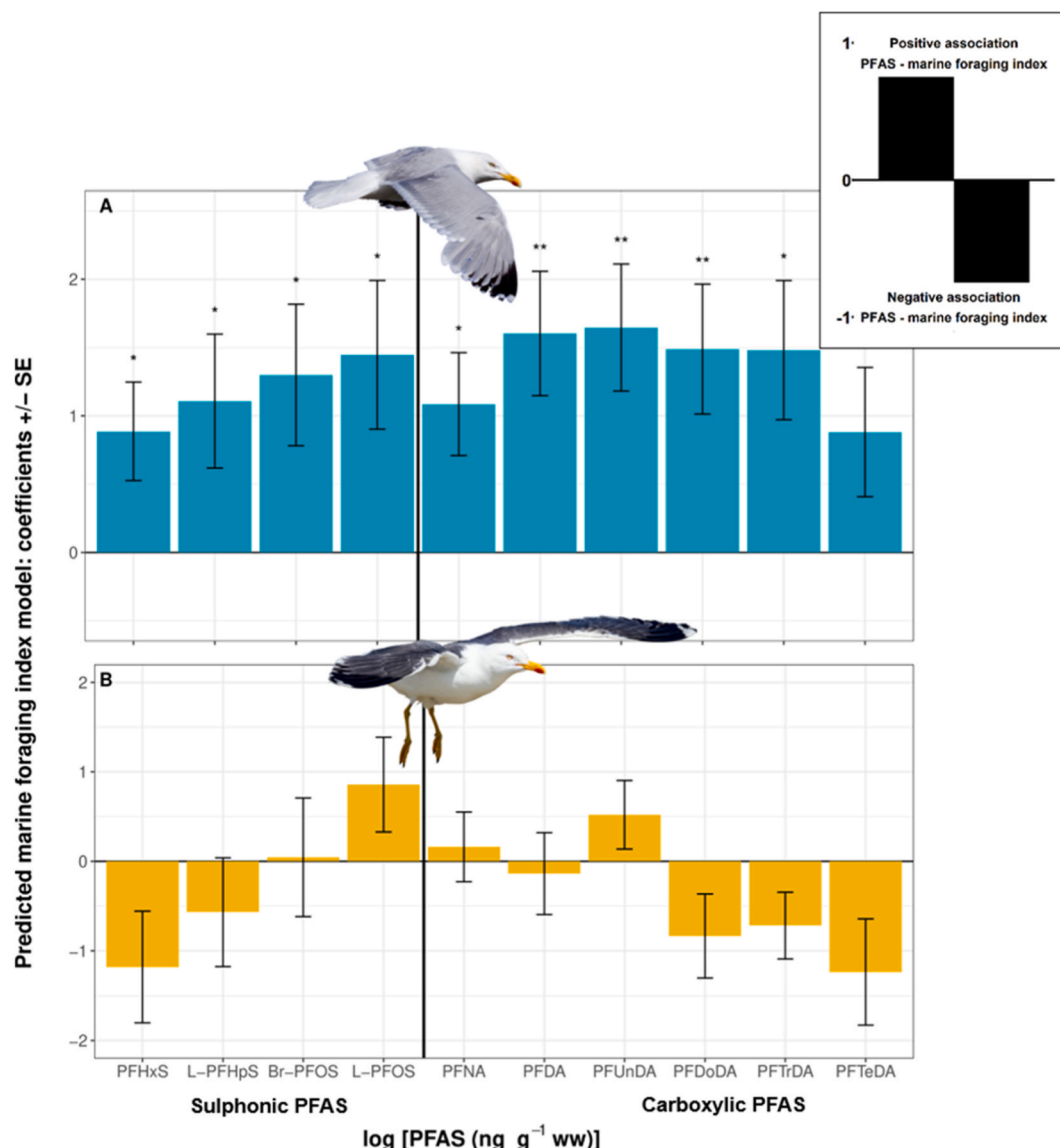
#### 4. Discussion

This study investigated multi-species associations between PFAS concentrations and foraging ecology using stable isotopes and GPS-tracking. Results showed species-specific associations between PFAS concentrations and foraging ecology inferred by the two approaches: stable isotopes and GPS tracking. Herring gulls showed an increase of PFAS concentrations with increasing use of the marine environment. Regarding lesser black-backed gulls and great black-backed gulls, relationships between PFAS concentrations and foraging ecology were compound- and sex-dependent, with a few negative associations

between specific compounds and stable isotope values. The different spatial distributions and habitat selection of the three gull species could explain contrasted associations between PFAS concentrations and the proxies of foraging ecology.

##### 4.1. Relationship between PFAS concentrations and foraging ecology

Herring gulls had PFAS concentrations positively associated with stable isotope values (mostly,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values), suggesting that consumption on high trophic prey of marine origin leads to a higher exposure to these PFAS. Herring gulls used a mix of terrestrial and coastal foraging habitats and interestingly individuals were more contaminated as they forage in more coastal environments. Semi-aquatic and marine species are often reported to be more contaminated by PFAS (Guckert et al., 2023), potentially related to

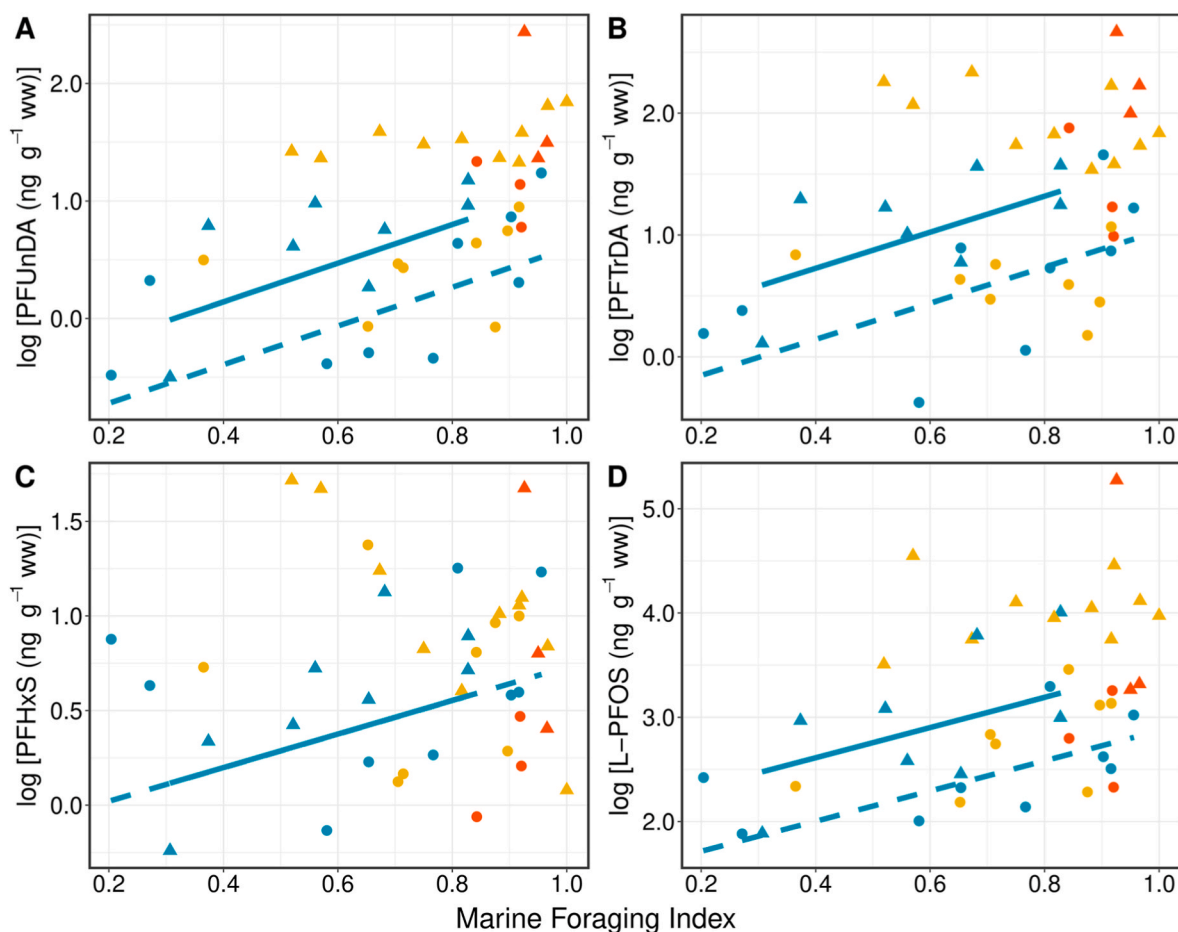


**Fig. 4.** Direction and significance of the marine foraging index coefficients in multivariate linear regressions to PFAS concentrations. A: herring gull (blue); B: lesser black-backed gull (yellow); Great black-backed gulls were not retained in this analysis due to low sample size ( $n = 6$ ). PFAS are classified by chemical structure (sulfonic and carboxylic) and ordered by carbon chain length. The height of each bar represents  $\beta_X$  of predicted linear coefficient of marine foraging index for PFAS X. If  $\beta_X > 0$  (resp.  $< 0$ ), then the concentration of PFAS X increases (resp. decreases) when the marine foraging index increases (resp. decreases) (see small panel). Error bars are standard errors. The stars represent the significance of the  $t$ -test comparing the difference of  $\beta_X$  to 0: \*:  $0.05 \geq p > 0.01$ ; \*\*:  $0.01 \geq p > 0.001$ ; \*\*\*:  $0.001 \geq p$ , and their absence means no significance. Note that the best model explaining variations in PFAS concentrations is for panel A: the model with a marginal sex effect for herring gulls and for panel B: the null model for lesser black backed gulls. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

bioconcentration, bioaccumulation, and biomagnification processes depending of the food webs (Conder et al., 2008; Hopkins et al., 2023; Kelly et al., 2024; Lewis et al., 2022). Specifically, coastal environment appear to present favorable conditions for the bioaccumulation and biomagnification of PFAS (Munoz et al., 2017a; Tansel, 2024), that could explain higher PFAS concentrations in gulls foraging in this environment. Trophic positions of the consumed preys probably differ in terrestrial and marine environments, as suggested by the positive correlation between the marine foraging index and  $\delta^{15}\text{N}$  values (Fig. S4.). Consequently, higher plasma PFAS concentrations in gulls could be explained by the consumption of higher trophic-level prey in the marine environment as compared to the terrestrial environment, as supported by higher PFAS concentrations when  $\text{PC}_1$  increases. Interestingly, significant associations between blood PFAS concentrations and foraging ecology were compound specific. Bioaccumulation and

biomagnification potentials of long-chain PFCAs could be one explanation of the positive associations between concentrations of these PFAS and stable isotope values. Previous studies have reported that the head functional group and carbon chain length play a significant role in PFAS biomagnification potentials, that increase for PFCAs with more than eight carbon atoms (Conder et al., 2008; Hopkins et al., 2023; Kelly et al., 2024; Lewis et al., 2022). Toxicokinetic (i.e., excretion, metabolism, organ distribution) of PFAS could also be compound-dependent and explain the differences found (Robuck et al., 2021).

For lesser black-backed gulls, the associations between blood PFAS concentrations and isotopic values were positives, negatives or non-significant. The positive associations between isotopic values and plasma concentrations of L-PFOS and PFUnDA suggest that individuals get contaminated by these compounds when they rely on marine resources, as suggested for herring gulls. Negative associations between



**Fig. 5.** Relationships between PFAS concentrations and the marine foraging index with four representative PFAS (two carboxylic PFAS and two sulfonic PFAS) chosen as an example. A: PFUnDA; B: PFTrDA; C: PFHxS, and D: L-PFOS as a function of proportion of marine foraging sites. Each point represents an individual. Lines are predicted significant linear relationships between PFAS concentrations and PC<sub>1</sub>. Colours indicate the species with European herring gull, lesser black-backed gull, and great black-backed gull in blue, yellow, and red, respectively. Raw values for great black-backed gull individuals have been plotted for information despite the fact that we did not fit any model to relate them to the marine foraging index. Line types and shape points are different for females and males, with dashed lines and circles for females and plain lines and triangles for males. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

PFHxS, L-PFHpS, PFDoDA or PFTeDA concentrations and stable isotope values suggested higher concentrations for lesser black-backed gulls relying on terrestrial habitats. In open seawaters, PFAS can also be diluted, transported, and transformed by physical, chemical, and biological processes (Ogunbiyi et al., 2024; Tansel, 2024), that could be one explanation of these negative associations. Moreover, terrestrial habitats use by lesser black-backed gulls are intensive agricultural areas, an activity where PFAS, including PFHxS and PFHpS, can be used as active pesticide ingredients (Donley et al., 2024). However, these positive and negative associations were not supported by any correlation of PFAS concentrations with the marine foraging index established from GPS-tracking, suggesting confounding factors involved or that statistical power may be too low. Half-lives of  $\delta^{13}\text{C}$ ,  $\delta^{34}\text{S}$ , and  $\delta^{15}\text{N}$  in blood ranged from 16 to 19 days, 10–20 days, and 9–25 days, respectively (Arneson and MacAvoy, 2005; Carleton et al., 2008; Lourenço et al., 2015; Carleton and Rio, 2005). Thus, stable isotopes represent the habitat use during the breeding period, reflecting a larger temporal window than GPS-tracking. This temporal mismatches could blur potential relationships between habitat use inferred by isotopic values or GPS-tracking and blood PFAS concentrations. However, stable isotope values were highly correlated with the marine foraging index that was derived from GPS-tracking data (all  $p < 0.01$ ; Fig. S4), confirming that stable isotopes are relevant indicators of recent habitat use.

The associations between PFAS concentrations and foraging ecology

for lesser black-backed gulls contrasted with those reported for the herring gulls. These interspecies differences could be explained by the different marine habitats used, herring gulls only forage in coastal habitats while lesser black-backed gulls foraged in both coastal and offshore habitats. Coastal environments can be contaminated by PFAS through river inputs (Schmidt et al., 2019; Tansel, 2024), including the Sèvre Niortaise, the Seudre or the Charente estuaries in the study area (Munsch et al., 2019, 2013; Serre et al., 2025). PFAS concentrations in seawater and sediments are reported to be influenced by physiochemical and oceanographic properties (i.e., salinity, organic matter, turbidity, and currents) that could lead to different contamination in coastal and offshore environments and the apex predators related to these marine foraging habitats (Li et al., 2024; Munoz et al., 2017b; Ogunbiyi et al., 2024). However, we did not detect any significant correlation between PFAS concentrations and the use of coastal vs. offshore habitats (Supplementary Information 4). According to GPS-tracks, herring gulls and lesser black-backed gulls foraged in different terrestrial habitats (urban and landfill areas and fields, respectively) that may explain the interspecies differences observed in PFAS concentrations and associations with trophic ecology proxies. Additional ecotoxicological studies of abiotic (water or soil) and other biotic matrices, including prey in the different habitats, appear necessary to better understand the distribution of PFAS in terrestrial, coastal, and marine habitats.

Great black-backed gulls at Isle of Ré use the coastal environments



and feed at high trophic level (Jouanneau et al., 2022; Fig. 3). Low inter-individual variability in isotopic values in this species could explain the absence of associations between isotopic values and blood concentrations of multiple PFAS. Interestingly, this coastal species showed high concentrations of PFCAs (Sebastiano et al., 2021), supporting further that the coastal environment could be a strong source of contamination for these compounds.

#### 4.2. PFAS concentrations and other intrinsic or extrinsic drivers

Our study highlighted that associations between PFAS and foraging ecology were species dependent. These interspecific variations seemed partially explained by the differences in use of marine resources, as we discussed earlier, and other drivers are also certainly involved. For example, the associations between blood PFAS concentrations and stable isotope values sometimes differed between males and females, especially for lesser black-backed gulls. Sex-specific foraging ecology could be one explanation, as females used more coastal habitats than males (Fig. 3B), which is consistent with a previous study on this species (Camphuysen et al., 2015). However, for the association between PFAS concentrations and the marine foraging index in lesser black-backed gulls, sex had a marginal effect, suggesting caution in driving conclusions. As previously proposed by Roscales et al. (2019), sexual and species differences in metabolic capabilities may also be a significant factor in the intra- and interspecies differences observed in PFAS concentrations, but the close taxonomic affiliation of the three species in our study suggests further investigations. Gulls are income breeders (Hobson, 2006), thus assessing PFAS burden during the incubation period should reflect recent and local exposure. In plasma, the half-life of PFAS varies from several days (13–20 days for PFOS and 11 days for PFOA in avian model species, Newsted et al., 2007; Yoo et al., 2009), to several months (125 days for PFOS in chickens), or even several years for humans (Dawson et al., 2023). PFCAs are often reported to show much shorter half-lives in the plasma compared to PFOS, related to efficient elimination (Drew et al., 2022; Yeung et al., 2009; Yoo et al., 2009). Consequently, depending on the type of PFAS, plasma concentrations can reflect recent dietary uptake, but also longer-term exposure as blood acts also as a significant reservoir for some long half-lived PFAS (Yeung et al., 2009; Yoo et al., 2009). PFAS concentrations in plasma could thus be influenced by the habitat use and the diet of gulls several months before the study (e.g., wintering period, Léandri-Breton et al., 2024). Herring gulls and great black-backed gulls in this colony were mostly considered as resident species, with only restricted migratory movements along the French Atlantic coasts during winter (unpublished data). On the contrary, the lesser black-backed gulls migrate further south on the Iberian Peninsula, reaching as far as North Africa (unpublished data) where they could be exposed to additional sources of PFAS and thus making relationships between PFAS concentrations and foraging ecology during the breeding season less apparent. Tracking winter migration and feathers sampling, representative of concentrations during moulting, could also provide useful temporal and spatial information to understand inter- and intra-species differences in PFAS contamination (Léandri-Breton et al., 2024).

#### 5. Conclusion

Our study shows that the concomitant use of stable isotope analyses and biologging can be useful to better understand inter- and intra-species differences in PFAS contamination and specifically suggests that high trophic position and the use of coastal foraging habitats, can lead to higher PFCAs contamination compared to terrestrial habitats. The results obtained also highlight that the influence of trophic ecology and foraging habitat on PFAS exposure in wildlife can differ between species, sexes, and compounds. Future studies mixing biologging and stable isotopes should include a variety of marine habitats (coastal, neritic, pelagic) and foraging tactics (diving, surface feeding) used both

during the breeding and wintering seasons, as PFAS distribution is likely to differ seasonally, between habitats, and the prey using these habitats.

#### CRediT authorship contribution statement

**David Rozen-Rechels:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Prescillia Lemesle:** Writing – review & editing, Writing – original draft, Visualization, Methodology. **William Jouanneau:** Writing – review & editing, Investigation, Formal analysis. **Manrico Sebastiano:** Writing – review & editing, Investigation. **Stephanie M. Harris:** Writing – review & editing, Formal analysis. **Pierre Blévin:** Writing – review & editing, Investigation. **Frédéric Angelier:** Writing – review & editing, Investigation. **Julien Gernigon:** Resources, Methodology, Investigation. **Jean-Christophe Lemesle:** Resources, Methodology, Investigation. **Frédéric Robin:** Writing – review & editing, Resources, Methodology, Investigation. **Hélène Budzinski:** Writing – review & editing, Resources. **Pierre Labadie:** Writing – review & editing, Resources. **Paco Bustamante:** Writing – review & editing, Resources. **Olivier Chastel:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

#### 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.2025.127556>.

#### Data availability

Data and scripts are publicly available on Zenodo (DOI: 10.5281/zenodo.17967553)

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