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Abstract

There is growing evidence that poly and perfluoroalkyl substances (PFAS) exposure leads to the disruption of thyroid hormones including thyroxine (T4) and triiodothyronine (T3), and may affect telomeres, repetitive nucleotide sequences which protects chromosome ends. Many seabird species are long-lived top predators thus exhibit high contaminant levels, and PFAS-disrupting effects on physiology have been documented especially in relation to the endocrine system in adults. On the contrary, studies on the developmental period (i.e., chicks), during which exposure to environmental contaminants may have a greater impact on physiological traits, remain scarce to this date. We carried out a multi-species study with the aim to assess whether and to which extent chicks of four gull species (herring gull, great and lesser black-backed gull, yellow-legged gull) in South Western France are contaminated by PFAS, and to bring further evidence about their potential physiological consequences. Linear PFOS showed concentrations of concern as it was generally more than 10 times higher than the other PFAS, and exceeded a threshold toxicity level (calculated from previous studies in birds) in almost all sampled chicks. Nonetheless, in herring gull male chicks, total T3 levels were significantly and negatively associated with perfluorodecanoate (PFDA) and perfluorododecanoate (PFDoDA) and positively associated with perfluorotetradecanoate (PFTeDA) in female chicks. Total T3 levels were also positively associated with PFDoDA in great black backed gull male chicks and with perfluorotridecanoate (PFTrDA) in lesser black backed gull chicks. In lesser and great black-backed gulls, both females and males showed significant negative associations between several PFAS and their body condition, and a positive association between telomere length and L-PFOS in the yellow-legged gull. These results corroborate previous findings and need to be further explored as they suggest that PFAS may interfere with the physiological status of chicks during the developmental period, potentially inducing long-lasting consequences.

Keywords: seabirds, contaminants, PFAS, telomeres, thyroid hormones, physiology

Introduction

Poly and perfluoroalkyl substances (PFAS) are a group of synthetic compounds that have been widely used over the past decades, especially considering their thermal and chemical stability, which enables them to be used in nonstick cookware, waterproof and stain resistant fabrics, and firefighting foam amongst many other commercial products (Wang et al., 2017). Although they were initially thought to be harmless substances, PFAS currently represent a major global health concern. Because of their high persistence in the environment (Cousins et al., 2022), their ubiquitous presence (Giesy and Kannan, 2001), and their bioaccumulation potential (at least for some PFAS; Conder et al., 2008), there is growing evidence regarding the toxicity of these compounds for both humans and animals (Sebastiano et al., 2020; Sinclair et al., 2020; Wang et al., 2017). PFAS are known to have a strong affinity for serum carrier proteins including albumin, transthyretin and thyroid binding globulin, thereby having endocrine-disrupting capabilities (Kar et al. 2017; Lihui et al. 2023). They can affect immunocompetence, disrupt the endocrine system, and impact on several physiological pathways in both humans and laboratory animals (DeWitt, 2015; Sunderland et al., 2019).

PFAS have been found globally in wildlife (Giesy and Kannan, 2001). It is increasingly recognized that the release of these chemicals into the environment is a major threat for wildlife and population viability, yet the consequences of PFAS exposure in wildlife remain poorly investigated. Determining how PFAS affect free-living vertebrates is challenging, but such compounds have been linked to a wide range of biological effects. One of the well-studied example of PFAS disrupting capacities lies with the competition of PFAS with thyroxine (T4) for binding to the thyroid hormone transport protein transthyretin (TTR). These disruptions may reduce circulating thyroid hormone levels including triiodothyronine (T3) and thyroxine (T4) (Kar et al., 2017; Ren et al., 2016). Indeed, the literature supports such thyroid-disrupting effect of the exposure to both old- and new-generation PFAS (Coperchini et al., 2020), but studies on these disrupting effects are underrepresented in wildlife.

The disruption of thyroid hormone levels in birds may be highly detrimental as they play a major role for development, behaviour, metabolism and reproduction (McNabb, 2007). In thick-billed murres *Uria lomvia*, the levels of free T3 (FT3) increased with increasing concentrations of certain PFAS, while total T3

(TT3) decreased with increasing PFAS levels, suggesting thyroid disruption (Choy et al., 2022). Recent work on the glaucous gull *Larus hyperboreus* found a positive association between perfluorooctanesulfonate (PFOS) and FT3 levels (Melnes et al., 2017). Similarly, adult great black backed gulls *Larus marinus* exposed to high PFAS levels showed a positive association between TT3 and several PFAS in females, but an opposite pattern was found in males (Sebastiano et al., 2021). PFAS were also positively associated with circulating thyroid hormones and thyroid gland activity in peregrine falcons *Falco peregrinus* chicks (Sun et al., 2021). These findings suggest a strong impact of PFAS exposure on the thyroid functioning and thyroid hormone regulation in birds. However, given the mixed results and the importance of optimal thyroid hormone levels in birds, this relationship needs to be more extensively investigated.

Telomere length and telomere dynamics (i.e. their variation in length over time) also represent potential physiological markers to investigate the toxicological consequences of PFAS on wildlife health. Telomeres are regions of repetitive DNA sequences which protect the ends of chromosomes and play a key role in aging and cell senescence (Aubert and Lansdorp, 2008). Their length represents a relevant measure of physiological stress in vertebrates (Angelier et al., 2018; Chatelain et al. 2020). In birds, telomeres are related to maximum lifespan (Tricola et al., 2018), reflect developmental stress (Boonekamp et al., 2014), and are an index of individual quality (Angelier et al., 2019), thus representing a key physiological marker to investigate the impact of contaminants on fitness (Louzon et al. 2019). Previous work in birds found that certain PFAS are positively associated with telomere length and telomere dynamics (Blévin et al., 2017a; Sebastiano et al., 2020). For instance, a positive relationship between PFASs and telomere dynamics was documented in black-legged kittiwakes *Rissa tridactyla,* with elongated telomere in birds bearing the highest PFAS concentrations (Blévin et al., 2017a). Similarly, glaucous gulls exposed to higher concentrations of certain PFAS showed the slowest rate of telomere shortening, and, in some individuals, telomere elongation (Sebastiano et al., 2020). However, no relationship was found between PFAS and telomere length in white-tailed eagle *Haliaeetus albicilla* chicks (Sletten et al., 2016). The physiological mechanisms through which PFAS disrupt key physiological processes and organism functioning need to represent a priority of ecotoxicological studies as this remains a largely unexplored area of research in wildlife.

Because many seabird species are long-lived top predators, they often exhibit high levels of persistent and biomagnifying contaminants (Rowe, 2008), and are considered as ideal models to assess the occurrence, levels and fate of contaminants in the environment. Seabird also aggregate in colonies during the reproductive season, offering the opportunity to investigate several species simultaneously and from the same geographic area, which can help us understand the factors driving intra- and inter-specific variation in contaminant exposure. So far, most of our knowledge about toxicological responses to contaminant exposure in seabirds relies upon studies conducted on adult birds. Comparatively much less is known on chicks as compared to adults, although detrimental consequences early in life are key determinant of the future fitness outcomes. Here, we carried out a multi-species study with the aim to assess to which extent seabird chicks of four seabird species from South Western France (European herring gulls *Larus argentatus*, lesser black-backed gulls *L. fuscus*, great black-backed gulls, and yellow-legged gulls *L. michahellis* are contaminated by PFAS. Previous work on the adults of the same species reported the presence of high concentrations of PFAS (e.g., range between 26.51 and 119.69 ng/g PFOS in lesser black-backed gulls; Sebastiano et al., 2021), with certain PFAS showing comparable or higher concentrations than seabird colonies known to be highly contaminated (Sebastiano et al., 2021). The combination of high levels of environmental contaminants and the simultaneous presence of several species in the same geographical location makes the study area ideal to explore the animals' response to environmental contamination in the wild. We therefore additionally investigated the relationship between exposure to PFAS and physiological endpoints including the levels of the plasma thyroid hormone T3 – i.e. the active from of thyroid hormones and telomere length in growing chicks. We expected to find similar association than those found in adults (mostly positive associations between TT3 and PFAS; Sebastiano et al. 2021), but the strength of these associations could be either reduced (due to the expected lower concentrations in chicks) or exacerbated (due to the increased sensitivity in chicks).

Materials and Methods

Sampling

Field work has been carried out from 2016 to 2019 at the Lilleau des Niges Natural Reserve (46° 13' 53" N, -1° 30' 22" W), managed by the Ligue pour la Protection des Oiseaux (LPO) located on the North side of Ile de Ré, France, as a part of a monitoring program for PFAS in the region. From 2016 to 2018, 21 European herring gulls *Larus argentatus* (n=10 in 2016, n=11 in 2018); 20 lesser black-backed gulls *L. fuscus* (n=10 in 2016, and n=10 in 2018); and 17 great black-backed gulls *L. marinus* (n=9 in 2016, and n=8 in 2018) chicks, were captured and sampled. In 2019, 12 yellow-legged gulls *L. michahellis* were additionally sampled, for a total of 70 chicks.

Chicks were captured by hand on their nests, when they were all the same age (approximately 1 month old). After capture, 2mL of blood was collected from the alar vein using a heparinized syringe and a 25-gauge needle. Blood was kept in a cold container in the field and centrifuged for 10 min at 8,000 x g at 20 °C at the laboratory within a few hours after collection to separate plasma and red blood cells, which were kept frozen at -20 °C until laboratory analyses. Skull and tarsus length were measured with an accuracy of 0.1 mm using a caliper. Wing length was also measured with an accuracy of 1 mm using a ruler, and birds were weighted to the nearest 5 g using a Pesola spring balance.

DNA was extracted from erythrocytes and the sex of the birds was determined at the CEBC ('Service d'Analyses Biologique') by polymerase chain reaction (PCR) amplification of two highly conserved genes (CHD) as described in (Fridolfsson and Ellegren, 1999)**.** Amplification was performed in 20µl final volume with an Eppendorf Mastercycler using 0.5 U Taq DNA polymerase, 200µM dNTPs, 10mM Tris-HCl pH 8.3, 50mM KCl, 1.5mM MgCl2 and 0.4µM of primers 2550F (5'- GTTACTGATTCGTCTACGAGA-3') and 2718R (5'-ATTGAAATGATCCAGTGCTTG-3').

Blood volume was too low to conduct laboratory analysis in one lesser black-backed gull chick. Therefore, a total of 69 chicks were included in the final dataset (21 European herring gull (n=10 in 2016, n=11 in 2018); 19 lesser black-backed gull (n=9 in 2016, and n=10 in 2018); 17 great black-backed gull (n=9 in 2016, and n=8 in 2018); and 12 yellow-legged gull chicks from 2019.

PFAS analyses

A total of 14 PFAS were analysed at the EPOC lab in each plasma sample using liquid chromatography coupled with tandem mass spectrometry (LC-ESI-MS/MS) on a 1290 LC system interfaced with a 6490 triple

quadrupole mass spectrometer operated in Multiple Reaction Monitoring mode (MRM; Agilent Technologies, Massy, France), including eight perfluoroalkyl carboxylic acids: branched- (Br-PFOA) and linear-perfluorooctanoate (L-PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorododecanoate (PFDoDA), perfluorotridecanoate (PFTrDA), perfluorotetradecanoate (PFTeDA); and six perfluoroalkyl sulfonic acids: perfluorohexanesulfonate (PFHxS), branched- (Br-PFHpS) and linear-perfluoroheptasulfonate (L-PFHpS), branched perfluorooctanesulfonate (Br-PFOS), linear perfluorooctanesulfonate (L-PFOS), and perfluorooctanesulfonamide (FOSA). Analytical standards of native PFAS along with a series of 10 ^{13}C, ^{18}O or D mass-labelled internal standards used for quantification purposes were supplied by Wellington laboratories. All reagents were analytical grade or equivalent and further information on recoveries can be found in Munoz et al. (2017). Analyte quantification was performed using six-point internal calibration (0.1-100 ng/g plasma equivalent). Accuracy was determined based on replicate analyses of chicken plasma samples spiked at 2 ng/g (n=15). A detailed protocol for the methodology used for analyzing the PFAS, quality assurance/ quality control (QA/QC) results, detection frequencies, accuracy, and LOD of all PFAS analyzed in the study can be found in the supporting information and in Table S1 and Table S2. Br-PFOA, L-PFOA, Br-PFHpS, and FOSA had a detection frequency below 40% of samples and were not included in the statistical analyses.

Thyroid hormone analyses

Total T3 was determined by radioimmunoassay at the CEBC (‘Service d’Analyses Biologique') in all samples collected until the breeding season of 2018, after which laboratory analyses were performed (therefore in 21 European herring gulls; 19 lesser black-backed gulls; 17 great black-backed gulls, but not in yellow legged gull samples collected later on in 2019). Briefly, 25 µL of plasma was incubated for 24h at 4 °C with a known concentration (10000 cpm) of T3 marked with the radioisotope Iodine-125 (T3-125I, Perkin Elmer, US, reference: NEX110X100UC) and an antibody Ab (polyclonal rabbit antiserum, Sigma-Aldrich, US, reference: T-2777). Because Ab is available in a limited concentration, T3 and T3-125I compete for Ab, to which they bind. Therefore, after incubation, there is a bound fraction (T3 and T3-125I bound to Ab) and a free fraction (T3 and T3-125I unbound to Ab), which are separated by adding a sheep anti-rabbit antibody (whole anti-serum anti rabbit IgG produced in sheep), incubated for 12h at 4 °C followed by centrifugation

178 at 4,300 x g at 18-20°C for 45 min. The bound fraction is then counted with a wizard 2 gamma counter
179 (Perkin Elmer, US). Pooled plasma of diverse gull samples was serially diluted and produced a dose-
180 response curve parallel to the T3 standard curve.
181 All samples had TT3 concentration above the minimum detectable concentration of 0.07 ng/ml
182 (LOD). All samples were run in duplicates. Samples that had a coefficient of variation above 12% between
183 the two duplicates were done in triplicates. The intra-assay coefficient of variation was 9.7%, while the
184 inter-assay coefficient of variation amounted to 15.1%.
185 Telomere analyses
186 Telomere length analyses were performed at the CEBC ('Service d'Analyses Biologique') in red blood cell
187 samples collected from 2016 to 2019, using a real-time quantitative polymerase chain reaction (qPCR)
188 technique as previously done (Sebastiano et al., 2020). Briefly, DNA was extracted from red blood cells
189 using DNeasy Blood and Tissue Kit (Qiagen), checked for quality and quantity with an optical density
190 spectrophotometer (Nanodrop ND-1000, Thermo Fisher Scientific, US) and by running DNA samples on a
191 gel. RAG1 (Recombination activating gene 1) gene was selected as our reference gene ("single copy gene").
192 The qPCR was performed with the telomere primers (Tel1b and Tel2b) and RAG1 primers (RAG1_F and
193 RAG1_R), using 2.5 ng of DNA per reaction. Telomere length is expressed relative to the single copy
194 reference gene (RAG1) measured on the same DNA sample (i.e., T/S ratio). Further clarifications on the
195 methodology used for telomere length estimation can be found in the Supporting information. One sample
196 could not be done in lesser black-backed gull chicks. A total of 67 samples - for which we had both telomere
197 length and PFAS (21 European herring gulls; 18 lesser black-backed gulls; 17 great black-backed gulls; 11
198 yellow-legged gull chicks from 2019) - were therefore available for further analyses.
199 Statistical analyses
200 First, we investigated whether PFAS concentrations showed significant differences between males and
201 females, or between the sampling year (2016 and 2018). We further tested whether any difference in
202 absolute PFAS concentrations occurred among the studied species (herring gulls, lesser and great black-
203 backed gulls) using linear models. Each PFAS was considered as a dependent variable while the factors
204 *Species*, *Year*, *Sex*, and their interactions (e.g. *Species:Sex*) were considered as predictors.

Second, we explored the association between i) TT3, ii) telomere length, and iii) the body condition (as dependent variables), and PFAS (as predictor) in samples collected in 2016 and 2018 using linear models. The body condition (hereafter body mass index *BMI*) has been calculated using the body mass adjusted by a coefficient generated by the relationship between the body mass and a linear body measurement (i.e. skull length) using the formula [body mass*mean skull length / skull length)^overall coefficient of the linear model between body mass and skull], as described in Peig and Green (2009). The skull length has been used because it is a very reliable and repeatable skeletal measurement and is highly correlated with body mass in all species (Pearson's correlation coefficient above 0.75 in each species). A three-way interaction term between PFAS and the factors *Species* and S*ex*, was used to investigate whether the association between the dependent variables (i.e., TT3, telomere length, and BMI, respectively) and PFAS could be related to the gender or was only occurring in a particular species. These models additionally included the *Year* of sampling (as a factor, to control for the temporal variation in total T3 and PFAS between 2016 and 2018), and the *Skull length* (as a covariate), to control for any age-related difference among chicks. The models on TT3 and telomere length also included the *BMI* (as a covariate, to control for the individual condition of birds). In these models, total T3 and PFAS were $\log_{10}$ transformed.

Because data on yellow-legged gulls were only collected in 2019, linear models for this species were done separately to test the association between telomere length and *BMI* (as dependent variables) and PFAS (as predictors). In all models on yellow-legged gulls, the factors *Sex*, *BMI* and the interaction between PFAS and *Sex* were included as predictors. The *Skull length* was included in the models on telomere length to control for any age-related difference among chicks. PFAS were $\log_{10}$ transformed.

Data transformation was done to meet model assumptions as homoscedasticity and normality of residuals, further confirmed by visually inspecting Q-Q plots (not achieved in the models in yellow-legged gulls likely due to the small sample size (n=11) and the high variation in concentrations for these contaminants). All data transformation and violation of models' assumptions are reported throughout the manuscript. Statistical significance was set to α=0.05 and 95% confidence intervals were used during data processing and data visualization. A complete list of the models used in the present study can be found in the supporting information. All statistical analyses were performed using R version 3.5.2.

Results

PFAS concentrations are summarized in Table 1 and Figure 1, and their detection frequency is reported in Table S1. ∑PFAS ranged from an average of 26.09 ng/g in males to 32.26 ng/g in females in herring gulls; an average of 34.95 ng/g in males to 37.84 ng/g in females in lesser black-backed gulls; an average of 26.77 ng/g in females to 28.80 ng/g in males in great black-backed gulls; and an average of 33.79 ng/g in males to 42.11 ng/g in females in yellow legged gulls. ∑PFAS was mostly represented by L-PFOS, which was the most abundant PFAS in all species (above 60% in all species, Table 1), having concentrations about 10 times higher than the other PFAS. Concentrations were as follows: in herring gulls L-PFOS > PFHxS > Br-PFOS > PFUnDA > PFNA > PFDA > PFTrDA > PFDoDA > PFTeDA > PFHpS; in lesser black-backed gulls L-PFOS > PFUnDA > Br-PFOS > PFHxS > PFTrDA > PFDA > PFNA > PFDoDA > PFTeDA > PFHpS; in great black-backed gulls L-PFOS > Br-PFPS > PFTrDA > PFUnDA > PFDA > PFHxS > PFDoDA > PFNA > PFTeDA > PFHpS; and in yellow-legged gulls L-PFOS > Br-PFOS > PFDA > PFUnDA > PFTrDA > PFNA > PFHxS > PFDoDA > PFTeDA > PFHpS. There were no significant differences between sexes in each species for all analysed PFAS (all $p \geq 0.07$, Table 1), nor between PFAS levels between 2016 and 2018 (all $t < 2.79$, all $p > 0.08$) except for PFHpS, which was higher in 2016 than 2018 in great black-backed gulls ($t = 3.15$, $p = 0.03$).

There were statistically significant differences among species for most carboxylic and sulfonic PFAS (Figure 1, Table S3). Among perfluoroalkyl carboxylic acids, PFUnDA was higher in lesser black-backed gulls than great black-backed gulls and herring gulls (both $t > 2.75$, both $p < 0.05$; Figure 1, Table S3); PFDoDA was higher in both lesser and great black-backed gulls than herring gulls (both $t > 3.24$, both $p < 0.01$; Figure 1, Table 3); PFTrDA and PFTeDA were higher in both lesser and great black-backed gulls than herring gulls (all $t > 3.41$, all $p < 0.01$; Figure 1, Table S3), and were also higher in great black-backed gulls than lesser black-backed gulls (both $t > 2.65$, both $p < 0.05$; Figure 1, Table S3). Among sulfonic acids, PFHxS was highest in herring gulls (both $t > 4.60$, both $p < 0.001$; Figure 1, Table S3); PFHpS was higher in herring gulls than great black-backed gulls ($t = 3.28$, $p < 0.01$; Figure 1, Table S3); and L-PFOS was highest in lesser black-backed gulls (both $t > 2.91$, both $p < 0.05$; Figure 1, Table S3). Yellow-legged gull chicks showed the highest average levels

of PFNA, PFDA, PFDoDA, Br- and L-PFOS when visually comparing them with the other species (Figure 1), although this was not statistically tested due to the different years of sampling.

TT3 was similar between 2016 and 2018 (all $t<2.04$, all $p>0.34$) and between males and females (all $t>1.38$, all $p>0.99$) in each species. In herring gull males, TT3 was significantly and negatively associated with PFDA and PFDoDA ($t=-2.40$, $p=0.02$ and $t=-2.00$, $p=0.05$, respectively; Figure 2, Table S4), and showed a non-significant tendency to be negatively associated with PFUnDA and PFTeDA ($t=-1.83$, $p=0.07$, and $t=-1.87$, $p=0.07$, respectively, Table S4). In herring gull females, TT3 was positively associated with PFTeDA ($t=2.26$, $p=0.03$; Figure 2, Table S4). TT3 was also positively associated with PFDoDA ($t=2.24$, $p=0.03$, Figure 2, Table S4) in great black-backed gulls, and showed a non-significant tendency to be positively associated with PFUnDA ($t=1.70$, $p<0.10$, Table S4), while it was positively associated with PFTrDA in lesser black-backed gulls independently of the sex of the birds ($t=2.06$, $p<0.05$; Figure 2, Table S4).

There was no association between telomere length and skull length in any of the study species (all $t<1.43$, all $p>0.18$). Telomere length was similar between 2016 and 2018 in all species (all $t<0.46$, all $p>0.99$, not tested in yellow-legged gulls for which we only had 2019 data), and between males and females (all $t<0.89$, all $p>0.39$) in all four species. Among the three species, telomere length showed a non-significant tendency to be negatively associated with PFDA, PFHpS, Br- and L-PFOS (all $t<-1.81$, all $p<0.10$; Table S5) in great black-backed gull chicks.

There was no difference in BMI between males and females (all $t<1.82$, all $p>0.46$) and between sampling years (all $t<0.45$, all $p>0.99$) in each species. BMI was similar among species (all $t=-2.01$, all $p>0.10$) and between sexes in yellow-legged gulls (run on separated models; both $t<0.89$ both $p>0.39$). BMI was negatively correlated with PFDA, Br-PFOS and L-PFOS in great black-backed gull females (all $t<-2.15$, all $p\leq0.04$, Figure 3, Table S6). In lesser black-backed gull males, BMI was negatively associated with L-PFOS ($t=-2.10$, $p=0.04$, Figure 3, Table S6) and showed a non-significant tendency to be negatively associated with Br-PFOS ($t=-1.90$, $p=0.06$, Table S6). BMI also showed a general negative association with PFNA and PFHpS independently of the sex of the birds and the considered species (both $t<-2.49$, both $p\leq0.02$, Figure 3, Table S6).

Separated models on yellow-legged gull chicks showed a significant positive association between telomere length and L-PFOS ($t=2.68$, $p=0.04$, Figure 4, Table S7), and a non-significant tendency to be positively associated with PFUnDA ($t=2.34$, $p=0.06$, Figure 4, Table S7). Separated models on yellow-legged gull chicks showed a significant negative association between BMI and PFUnDA ($t=-3.14$, $p=0.01$, Figure 4, Table S8), while this association was non-significant with the other PFAS (all $t<1.77$, all $p>0.11$, Table S8).

Discussions

Our study investigated the potential disrupting effects of PFAS on body condition, thyroid hormone levels, and telomere length of chicks from four seabird species from South Western France. By comparing PFAS concentrations in chicks with those found in the literature from other species and regions, we found that some chicks are exposed to high concentrations of PFAS. We also found differences in the contamination patterns among the species, possibly due to the different diet the young receive from their parents. In lesser and great black-backed gulls, both females and males showed significant negative associations between several PFAS and their body mass index, indicating that PFAS may interfere with chicks' development. Our results also corroborate the hypothesis that PFAS may impact on the physiological status of seabirds, as there were significant associations between several PFAS and the plasma concentrations of TT3 (both negative and positive depending on the studied species), and a positive association between telomere length and L-PFOS in the yellow-legged gull.

Our study is amongst the first to report the concentrations of PFAS in chicks of several seabird species from Europe. Among perfluoroalkyl carboxylic acids, the odd carbon numbered PFUnDA and PFTrDA were the most abundant, a pattern commonly found in other species as the glaucous gull (Melnes et al., 2017) and the kittiwakes (Tartu et al., 2014) from the Arctic. The levels of perfluoroalkyl carboxylic acids found in this study (ranging from a mean of 0.2 ng/g of PFTeDA in herring gulls to 2.1 ng/g of PFUnDA in great black-backed gulls) are generally lower than what has been found in adult birds from the same study area (ranging from a mean of 0.6 ng/g of PFNA in herring gulls to a mean of 6.9 ng/g of PFTrDA in great black-backed gulls; Sebastiano et al. 2021). These concentrations are similar to PFAS in a population of flamingo *Phoenicopterus roseus* chicks from the Ebro Delta in Spain (except for PFOA, which showed an

311 average concentration of 38.5 ng/mL; Dulsat-Masvidal et al., 2023), and lower than black-legged kittiwake
312 adults from Svalbard (ranging from a mean of 1.0 ng/g of PFNA to 18.2 ng/g of PFTrDA; Tartu et al., 2014).
313 Comparably to carboxylic acids, the concentration of perfluoroalkyl sulfonic acids in chicks reflected what is
314 often found in seabird studies, with L-PFOS concentrations representing over 65% of the total PFAS
315 concentration, followed by Br-PFOS, PFHxS, and PFHpS. As food represents the main pathway of exposure
316 to environmental contaminants in seabirds (Lavoie et al., 2013), chicks largely reflects the accumulation
317 through the food brought by the parents, showing contaminant concentrations that are usually lower than
318 those found in adult birds because the temporal window for bioaccumulation is much shorter (Sebastiano
319 et al., 2016; Sebastiano et al., 2017). While all species showed highest L-PFOS concentrations followed by
320 Br-PFOS, PFHxS, and PFHpS, reflecting the results on adult birds (Sebastiano et al., 2021), herring gull chicks
321 surprisingly exhibited higher levels of PFHxS in comparison with the other species, concentrations that are
322 also higher than what has been found in herring gull adults from the region (Sebastiano et al., 2021). As
323 bird eggs commonly contain very low level of PFHxS compared with other PFAS (Groffen et al., 2017;
324 Jouanneau et al., 2022a; Verreault et al., 2005), one possible explanation might be that the other species
325 fed their young with a higher proportion of eggs than herring gulls have, resulting in lower levels of blood
326 PFHxS. However, it also should be noted that previous work on the same seabird populations found that
327 the isotopic niche of chicks (inferred from nitrogen and carbon stable isotopes), highly overlapped among
328 the four studied species (Jouanneau et al. 2022b). Therefore, except the observed small inter-individual
329 variation in the way chicks are fed by their parents (Jouanneau et al. 2022b), other factors like excretion
330 and/or assimilation mechanisms, may also drive the observed concentrations.
331 As stated above, L-PFOS was the PFAS showing concentrations of concern (from ~17ng/g to
332 ~30ng/g average PFOS concentration depending on the species), similar to those found in adult great black-
333 backed gulls from Norwegian populations (Bustnes et al., 2008), and lower than those found in the
334 Audouin's gull *Larus audouinii* and yellow-legged gull from the Ebro Delta in Spain (from ~25ng/g to
335 ~61ng/g average PFOS concentration in adults; Bertolero et al., 2015). Such concentrations are also several
336 times lower than those reported in the lesser black-backed gull chicks samples 50 km away from a
337 fluorochemical plant in Antwerp, Belgium (average of 160 ng/mL in 4-week-old chicks; Lopez-Antia et al.,

2021), and from bald eagle *Haliaeetus leucocephalus* chicks from upper Midwestern United States located closely to a 3M fluorochemical plant (averages of different sampling sites ranging from 77 ng/mL to 800 ng/mL; Route et al., 2014). Although there are limited toxicity data for fluorinated compounds in birds, previous work in northern bobwhites quail *Colinus virginianus* estimated a LOAEL (lowest observed adverse effect level) of 11.6 ng/g of PFOS in liver samples of juveniles (Dennis et al. 2022). Considering a liver to whole-blood ratio of 2.72 for PFOS (as previously documented in chicks of several seabird species; Robuck et al. 2021), and assuming that plasma is about 50% of whole-blood (and therefore a liver to plasma ratio of 1.36 for PFOS), almost all sampled chicks exceed such 11.6 ng/g threshold effect. Deriving toxicity data from other studies (and from unrelated species) is, however, highly debatable since the effects are often species-specific (Sebastiano et al. 2022), thus these comparisons should be interpreted with caution. In birds - and more specifically, seabirds - the availability of toxicity data for many PFAS alone, and in combination with each other, remains very limited so far, thus we encourage future studies to seek to clarify this aspect.

The majority of studies that have investigated the presence of PFAS have been often carried out on adult birds, so these concentrations are also difficult to place in an ecotoxicological context as several other environmental factors, the health status of birds, and the concomitant exposure to other contaminants may enhance the susceptibility of individuals to a specific stressor (Sebastiano et al., 2022). Therefore, some PFAS may be associated with adverse health effects even when they occur at a lower concentration than other PFAS. Our results show that herring gull females with higher levels of PFTeDA have higher TT3 concentrations. Similarly, PFDoDA and PFTrDA were positively associated with TT3 in great black-backed gull males and lesser black-backed gulls, respectively. These positive associations with thyroid hormones are similar to what has been found in glaucous gull adult females (PFOS and free triiodothyronine (FT3); Melnes et al., 2017), in northern fulmar *Fulmarus glacialis* chicks (PFHpS, PFOS, PFNA, and total thyroxin (TT4); Nøst et al., 2012), and adult kittiwakes (L-PFOS, PFDA, and TT4 in males, PFDoDA, PFTrDA, PFTeDA and TT3 in females; Ask et al., 2021) from Svalbard, and in adult great black-backed gull females from France (PFUnDA, PFDoDA, PFTrDA, PFTeDA and Br-PFOS and TT3; Sebastiano et al., 2021). This positive association may be the result of an alteration of the hypothalamic-pituitary-thyroid (HPT) axis with the

consequent up-regulation of thyroid hormone receptors (reviewed in Coperchini et al., 2020). However, we also found that herring gull chicks exposed to higher concentrations of PFDA and PFDoDA showed the lowest TT3 concentration. To the best of our knowledge, this is the first study to report a negative association between PFAS and thyroid hormones in chicks, as such associations were only recently reported in adult great black-backed gull males (PFHxS and TT3; Sebastiano et al., 2021) and thick-billed murre males (PFOS, PFDoA, PFTeDA, and TT3; Choy et al., 2022). The maintenance of optimal levels of thyroid hormones is of fundamental importance for development (McNabb, 2007), and an interference in the HPT axis, which regulates the thyroid hormone cascade, could cause lasting effects in affected individuals (McNabb, 2007). Previous work in rodents showed that PFAS (i.e. PFHxS) can indeed induce developmental toxicity though a reduction in thyroxin levels (Ramhøj et al., 2018). In humans and laboratory studies on cell cultures, PFAS are known to interfere with the endocrine system and to reduce the production of T3 and T4, for instance, by depressing iodide peroxidase activity or by inhibiting iodine accumulation in thyroid cells (reviewed in Coperchini et al., 2020). Despite the recent findings suggesting a possible disruption of thyroid hormone levels in birds and other animals, our understanding of the underlying mechanisms remains very limited although it should represent a current priority. Because PFAS and thyroid hormones bind with plasma proteins (e.g. albumin; Forsthuber et al. 2020; McNabb 2007), the associations we found between TT3 and specific PFAS may also be associated with a difference in plasma protein concentrations in the studied birds. Birds with low plasma protein concentrations would therefore have low concentrations of both PFAS and thyroid hormones, and vice versa. Information on the free fraction of thyroid hormones in the plasma would also be highly beneficial in the present study to assess the importance of binding site competition between PFAS and thyroid hormones as an explanation for the observed associations. It also remains unclear why males and females seem to respond differently to the same stimulus even in chicks, as in our study, i) females showed similar levels than males in terms of absolute PFAS concentrations; ii) chicks do not show sexual dimorphism, a condition which in adults may affect their physiological status and their response to contaminant exposure; and iii) one of the sexes may be more susceptible at certain life stages in adults (e.g. reproduction in females, which is highly costly, Hanssen et al., 2005), which does not, however, apply to chicks.

Despite the known importance of studying telomere length in relation to contaminant levels in wild animals (Louzon et al. 2019), until a few years ago we knew almost nothing about the possible association between PFAS and telomeres. Sea eagle chicks failed to show any association (Sletten et al., 2016), but a positive association between PFAS and telomeres was found in kittiwakes (Blévin et al., 2017a) and in the glaucous gull (Sebastiano et al. 2020). In the present study, despite the relatively small sample size in yellow-legged gulls, we found a positive association between L-PFOS levels and telomere length, suggesting that chicks with the highest L-PFOS concentrations also have longer telomeres. One possible explanation for the positive associations between PFAS and telomere length (or the change in telomere length over time) may be related to the up-regulation of the activity of the telomerase, the enzyme responsible for maintenance of the length of telomeres. For instance, if PFAS impact on the hormones that regulate the activity of telomerase (directly by modulating its activity, or by decreasing the concentration of telomerase-inhibitory hormones), higher PFAS concentrations would result in longer telomeres, although evidence of such association is lacking. One alternative explanation for this PFAS - telomere association may lie in the nutritional status of the birds. Although yellow-legged gull adults from the study area show a generalist diet (Jouanneau et al., 2022b), the chicks included in this study are fed with high trophic level food of marine origin as highlighted by the high carbon and sulphur stable isotope values (Jouanneau et al., 2022b). If the high L-PFOS concentrations have no negative effect on the chicks of this species (given the absence of negative association with other markers), the high-quality diet they receive may actually have a beneficial effect on their physiology, including telomere length. This hypothesis needs to be specifically tested as it would suggest that under certain conditions (i.e., environmental contamination not being excessively elevated or species being particularly tolerant), the potential detrimental effects of PFAS exposure through food is negligible compared to the benefits of a high trophic level diet.

This potential positive association between food quality and telomere length is, however, contrasted by the results of the association between PFAS and body condition. The general negative association between the body condition of chicks and PFNA and PFHpS, together with the negative associations specifically found with PFDA, Br-PFOS, and L-PFOS in female great black-backed gull, with L-PFOS in male lesser black-backed gull, and with PFUnDA in yellow-legged gull, strongly suggest that

419 exposure to PFAS impacts on growth of developing seabirds. PFAS may alter the expression of genes
420 involved in the metabolism of lipids and fatty acids (Jacobsen et al., 2018) and induce adipogenesis (Xu et
421 al., 2016). Although our results are not in line with those found previously in male kittiwakes (Tartu et al.,
422 2014), they overlap with what has been found in adult birds of the same species from the same geographic
423 area (Sebastiano et al., 2021). Furthermore, it is also necessary to take into account that PFASs can affect
424 several physiological mechanisms as altering the levels of thyroid and steroid hormones (Coperchini et al.,
425 2020; Liu et al., 2020), thereby impacting on energy expenditure and metabolism (Blévin et al., 2017b).
426 Considering that those results were found in almost all studied species, and were all showing the same
427 pattern, we suggest that exposure to PFAS early in life may ultimately reduce the body condition of birds.
428 Given that we have limited understanding on this relationship, especially in developing birds, and given that
429 the directions of these associations appear to be species-specific, this aspect clearly warrants further
430 investigation.
431
432 **Conclusions**
433 Our study provides the first data on PFAS levels in chicks of several seabird species from Europe, during
434 their developmental period. Some PFAS showed comparable levels to other seabird species and lower
435 levels than adult birds from the same species and the same study area. However, in lesser and great black-
436 backed gulls, both females and males showed significant negative associations between several PFAS and
437 their body mass index, indicating that PFAS may impact on the health status of exposed individuals and
438 therefore pose a threat to long-lived seabirds. The hypothesis that PFAS may impact on the physiological
439 status of seabirds is further corroborated by the significant associations between PFAS and the plasma
440 concentrations of TT3 and telomere length (both negative and positive depending on the studied species).
441 These results assume even greater importance considering that they were found in chicks, and
442 need to be seriously considered as PFAS may interfere with their physiological status during the
443 developmental period and cause long-lasting consequences. We stress once again how even low
444 contaminant concentrations may be associated with a physiological response in animals, and that each
445 species or population reacts differently to stimuli. There is now a large body of evidence showing

associations between PFAS and physiological disruption in a variety of organisms, including seabirds. The experimental investigation of the mechanisms through which PFAS interfere with physiological and organism functioning need to represent a priority of ecotoxicological studies as this remains a largely unexplored area of research in wildlife.

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Table 1: Plasma PFAS concentrations (ng/g of ww) in females and males of the four gull species from South Western France. The % refers to the percentage of contribution of each PFAS, while t- and p-values refer to the difference in PFAS between females and males. Significant p-values are bolded.

	Females			Males				
	mean ± SE	**median (range)**	**%**	**mean ± SE**	**median (range)**	**%**	**t-value**	**p-value**
Herring gull *Larus argentatus*								
PFNA	1.19 ± 0.07	1.17 (0.67 - 2.02)	3.7%	0.90 ± 0.01	0.86 (0.45 - 1.22)	3.5%	1.57	0.62
PFDA	1.09 ± 0.05	1.04 (0.64 - 1.63)	3.4%	0.96 ± 0.02	0.95 (0.49 - 1.73)	3.7%	0.83	0.96
PFUnDA	1.59 ± 0.11	1.41 (0.99 - 3.28)	4.9%	1.30 ± 0.02	1.27 (0.70 - 1.80)	5.0%	1.27	0.80
PFDoDA	0.60 ± 0.02	0.51 (0.47 - 0.83)	1.9%	0.63 ± 0.01	0.60 (0.39 - 0.92)	2.4%	-0.17	0.99
PFTrDA	0.78 ± 0.02	0.72 (0.70 - 1.03)	2.4%	0.79 ± 0.01	0.74 (0.45 - 1.03)	3.0%	0.01	0.99
PFTeDA	0.20 ± 0.01	0.22 (0.13 - 0.27)	0.6%	0.23 ± 0.00	0.21 (0.16 - 0.33)	0.9%	-0.85	0.96
PFHxS	2.46 ± 0.18	2.14 (0.98 - 4.16)	7.6%	2.36 ± 0.04	2.23 (1.45 - 3.20)	9.1%	-0.08	0.99
PFHpS	0.36 ± 0.02	0.40 (0.19 - 0.51)	1.1%	0.30 ± 0.01	0.33 (0.12 - 0.41)	1.2%	1.16	0.85
Br-PFOS	1.96 ± 0.10	1.80 (1.19 - 2.91)	6.1%	1.58 ± 0.02	1.63 (1.00 - 2.12)	6.0%	1.71	0.72
L-PFOS	22.02 ± 1.29	20.28 (12.20 - 36.38)	68.2%	17.04 ± 0.34	17.96 (9.20 - 24.64)	65.3%	1.60	0.61
∑PFAS	32.26 ± 1.78	29.27 (18.54 - 49.98)		26.09 ± 0.41	28.10 (14.61 - 34.66)		1.41	0.72
Lesser black-backed gull *Larus fuscus*								
PFNA	1.18 ± 0.05	1.13 (0.68 - 1.67)	3.1%	0.99 ± 0.03	0.99 (0.43 - 1.66)	2.8%	0.08	0.99
PFDA	1.25 ± 0.06	1.22 (0.70 - 2.23)	3.3%	1.01 ± 0.03	1.02 (0.47 - 1.42)	2.9%	0.23	0.99
PFUnDA	2.05 ± 0.07	1.85 (1.39 - 3.01)	5.4%	1.98 ± 0.06	1.96 (1.13 - 3.01)	5.7%	0.28	0.99
PFDoDA	0.94 ± 0.04	0.91 (0.47 - 1.59)	2.5%	0.81 ± 0.02	0.80 (0.44 - 1.17)	2.3%	0.22	0.99
PFTrDA	1.13 ± 0.05	1.02 (0.57 - 1.91)	3.0%	1.19 ± 0.04	1.15 (0.58 - 1.80)	3.4%	-0.27	0.99
PFTeDA	0.36 ± 0.02	0.34 (0.18 - 0.59)	0.9%	0.28 ± 0.01	0.28 (0.15 - 0.42)	0.8%	0.84	0.96
PFHxS	1.67 ± 0.03	1.67 (1.35 - 2.00)	4.4%	1.45 ± 0.05	1.39 (0.69 - 2.54)	4.1%	0.19	0.99
PFHpS	0.28 ± 0.01	0.27 (0.21 - 0.40)	0.7%	0.27 ± 0.01	0.24 (0.14 - 0.48)	0.8%	0.20	0.99
Br-PFOS	1.89 ± 0.06	1.74 (1.37 - 2.75)	5.0%	1.87 ± 0.06	1.83 (0.97 - 3.37)	5.4%	0.50	0.98
L-PFOS	27.09 ± 0.98	27.11 (16.23 - 40.40)	71.6%	25.10 ± 0.80	22.25 (13.52 - 42.43)	71.8%	0.14	0.99
∑PFAS	37.84 ± 1.34	37.00 (23.31 - 56.23)		34.95 ± 1.01	32.85 (19.07 - 56.45)		0.15	0.99
Great black-backed gull *Larus marinus*								
PFNA	0.75 ± 0.03	0.73 (0.58 - 0.96)	2.8%	0.96 ± 0.04	0.80 (0.46 - 1.63)	3.3%	-1.06	0.89
PFDA	1.00 ± 0.04	0.96 (0.78 - 1.37)	3.7%	1.50 ± 0.09	1.21 (0.44 - 3.48)	5.2%	-1.79	0.48
PFUnDA	1.49 ± 0.04	1.56 (1.17 - 1.73)	5.6%	1.51 ± 0.04	1.52 (0.88 - 2.05)	5.2%	-0.10	0.99
PFDoDA	0.96 ± 0.02	0.96 (0.83 - 1.14)	3.6%	0.98 ± 0.03	0.88 (0.61 - 1.68)	3.4%	-0.12	0.99
PFTrDA	1.80 ± 0.05	1.77 (1.48 - 2.34)	6.7%	1.51 ± 0.05	1.43 (0.96 - 2.44)	5.2%	1.41	0.72
PFTeDA	0.42 ± 0.01	0.44 (0.34 - 0.50)	1.6%	0.38 ± 0.01	0.33 (0.24 - 0.67)	1.3%	0.84	0.96
PFHxS	1.41 ± 0.03	1.44 (1.11 - 1.65)	5.3%	1.46 ± 0.04	1.55 (0.59 - 2.08)	5.1%	0.53	0.99
PFHpS	0.22 ± 0.01	0.21 (0.14 - 0.33)	0.8%	0.24 ± 0.01	0.23 (0.12 - 0.45)	0.8%	-0.75	0.97
Br-PFOS	1.65 ± 0.05	1.59 (1.27 - 2.03)	6.2%	1.76 ± 0.06	1.75 (0.79 - 3.22)	6.1%	-0.57	0.99
L-PFOS	17.06 ± 0.62	17.11 (12.20 - 22.19)	63.7%	18.50 ± 0.74	18.91 (8.02 - 36.14)	64.2%	-0.62	0.99
∑PFAS	26.77 ± 0.77	27.06 (21.32 - 32.91)		28.80 ± 0.97	30.03 (14.01 - 51.24)		-0.61	0..99
Yellow-legged gull *Larus michahellis*								
PFNA	1.25 ± 0.05	1.17 (1.03 - 1.58)	3.0%	1.10 ± 0.06	1.19 (0.44 - 1.62)	3.3%	0.86	0.41
PFDA	2.00 ± 0.07	2.16 (1.51 - 2.36)	4.8%	1.94 ± 0.12	1.82 (0.95 - 3.18)	5.7%	0.47	0.65
PFUnDA	2.07 ± 0.12	1.92 (1.32 - 2.84)	4.9%	1.67 ± 0.03	1.64 (1.40 - 2.11)	5.0%	1.45	0.18
PFDoDA	1.15 ± 0.04	1.15 (0.86 - 1.38)	2.7%	0.93 ± 0.02	0.99 (0.63 - 1.10)	2.8%	2.00	0.07*
PFTrDA	1.72 ± 0.12	1.76 (0.83 - 2.47)	4.1%	1.36 ± 0.07	1.36 (0.62 - 1.97)	4.0%	1.02	0.33
PFTeDA	0.45 ± 0.02	0.49 (0.29 - 0.53)	1.1%	0.33 ± 0.01	0.34 (0.15 - 0.44)	1.0%	1.76	0.11
PFHxS	1.32 ± 0.05	1.21 (1.10 - 1.70)	3.1%	0.97 ± 0.04	1.14 (0.39 - 1.24)	2.9%	1.76	0.11
PFHpS	0.24 ± 0.01	0.24 (0.17 - 0.31)	0.6%	0.19 ± 0.01	0.20 (0.13 - 0.25)	0.6%	1.85	0.09
Br-PFOS	2.49 ± 0.13	2.41 (1.70 - 3.41)	5.9%	2.29 ± 0.16	1.93 (1.28 - 4.62)	6.8%	0.65	0.53
L-PFOS	29.41 ± 2.44	26.26 (15.98 - 44.45)	69.8%	22.99 ± 0.76	21.26 (18.09 - 32.22)	68.1%	1.04	0.32
∑PFAS	42.11 ± 2.93	38.47 (26.17 - 60.42)		33.79 ± 0.95	30.94 (25.97 - 45.23)		1.15	0.28

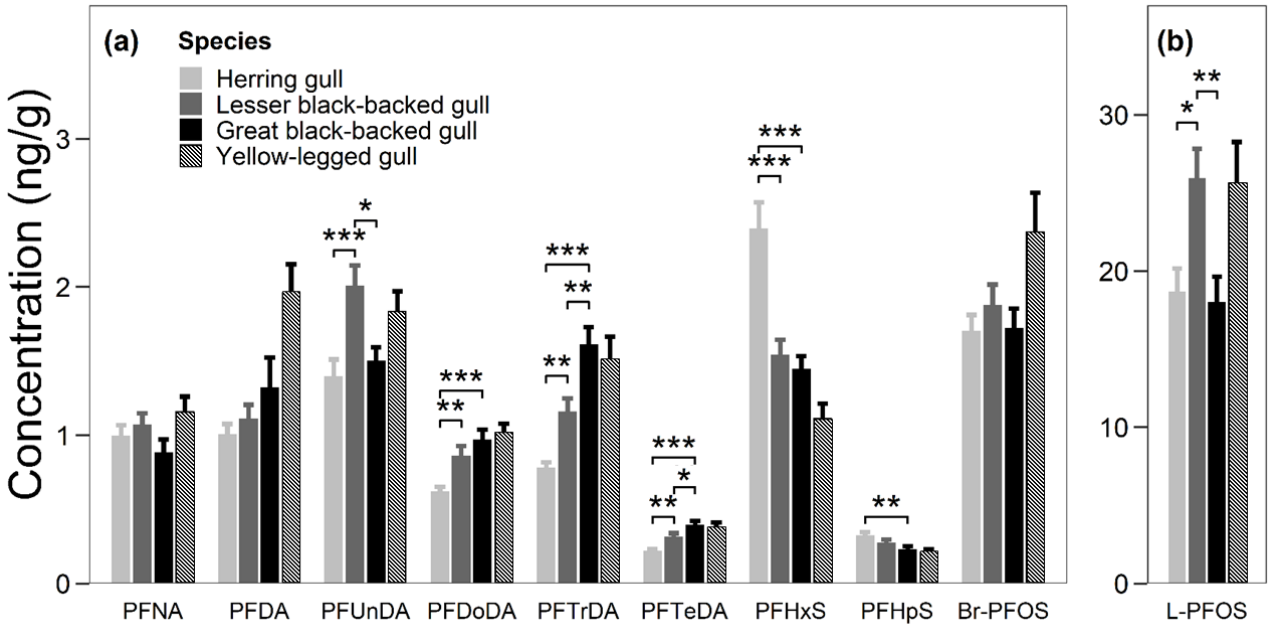
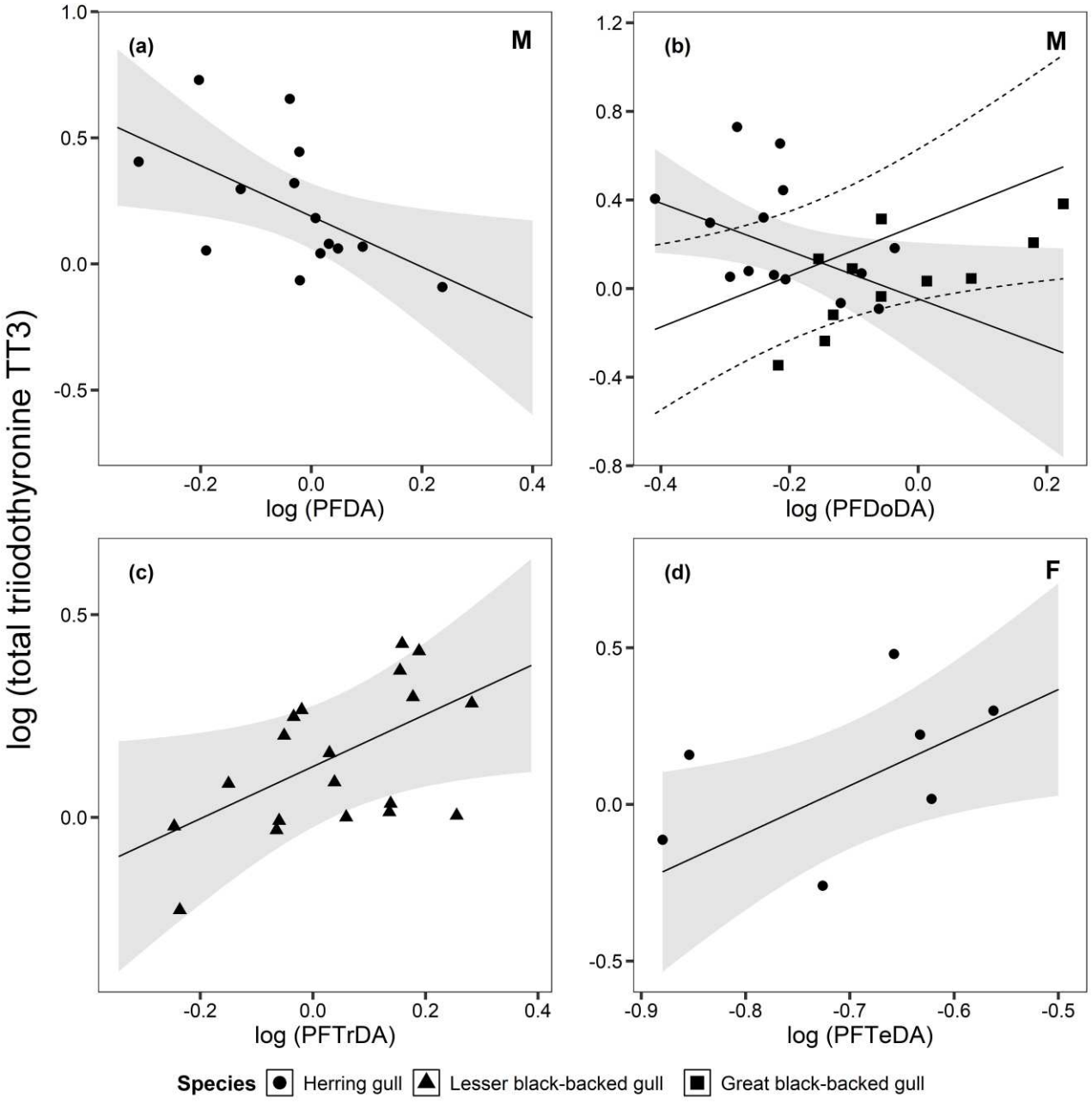


Figure 1. Plasma concentrations of PFAS (expressed as ng/g of ww) in the four gull species from South Western France. Statistically significant differences are indicated by the asterisks *, **, ***, which indicate a p-value < 0.05, <0.01, and <0.001, respectively. Yellow-legged gulls were sampled in a different year (2019) than the other species (2016 and 2018), thus statistical comparisons with the other species were not carried out.



480

481 **Figure 2.** Relationship between the concentration of the log-transformed TT3 and PFAS (PFDA, PFDoDA,
482 PFTrDA, PFTeDA; panel a to d, respectively), of the three seabird species from Ile de Re. Shapes of data
483 points are used to distinguish each species, and the solid line represents the trend. When more than a
484 single significant relationship occurs, polygons (representing 95% confidence intervals) following the first
485 one are bounded by a dotted line. Data refer to the period 2016 and 2018 for which both TT3 and PFAS
486 were available (n = 57).

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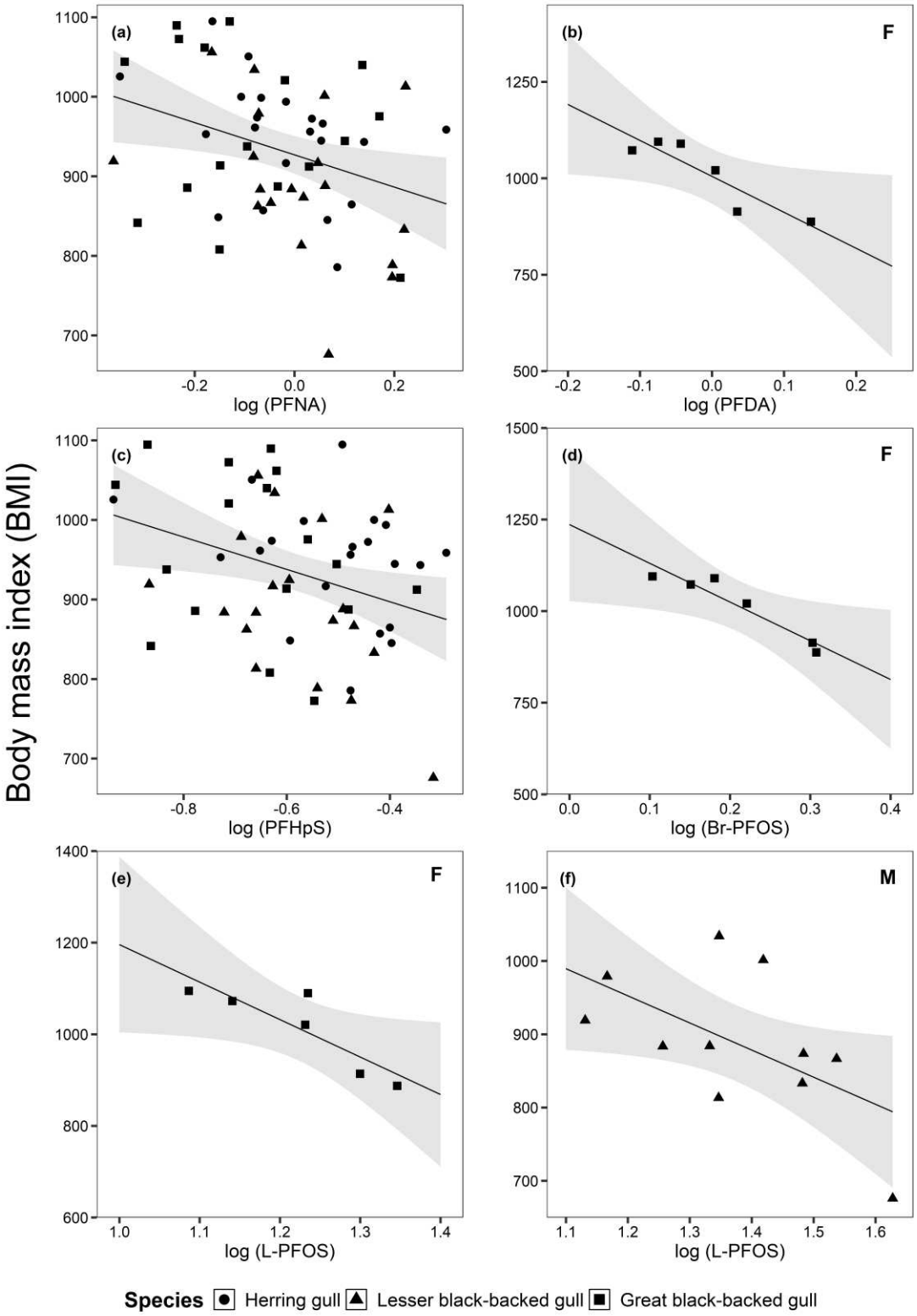

489

490 **Figure 3.** Relationship between the body mass index BMI and PFAS (PFNA, PFDA, PFHpS, Br-PFOS, and L-
491 PFOS; panel a to f, respectively), of the three seabird species from Ile de Re. Shapes of data points are used
492 to distinguish each species, and the solid line represents the trend. Polygons represent 95% confidence
493 intervals. Data refer to the period 2016 and 2018 for which both BMI and PFAS were available (n = 58).

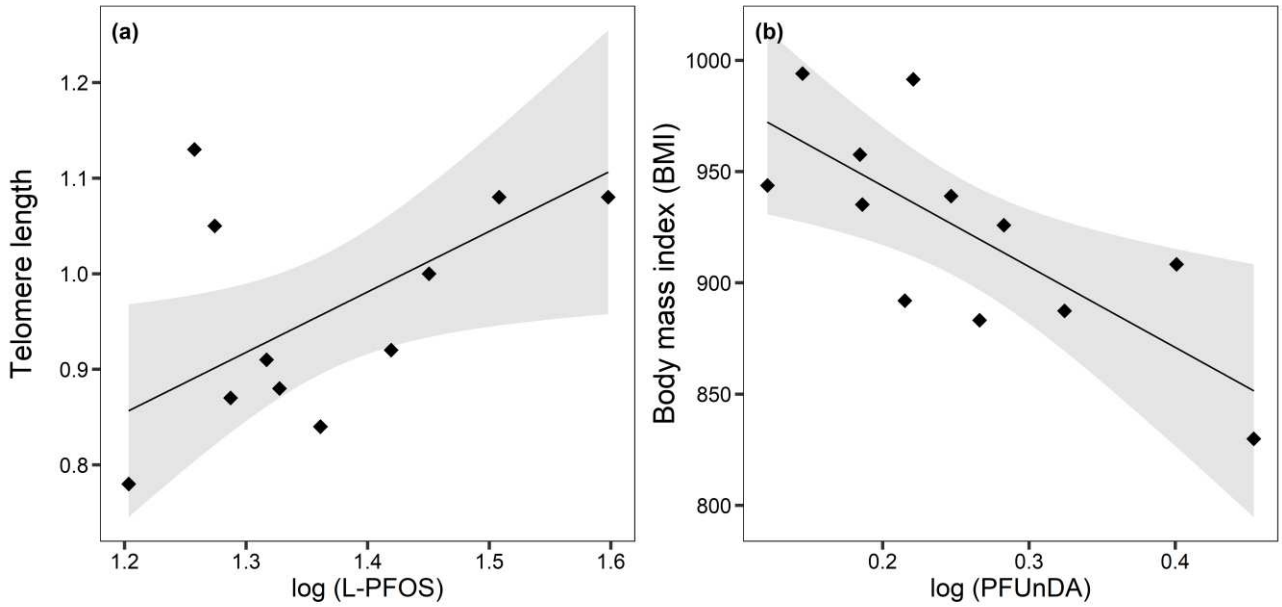

Figure 4. Relationship between (a) telomere length and L-PFOS and (b) BMI and PFUnDA in yellow-legged gull chicks from Ile de Re. The solid line represents the trend while the polygon represents 95% confidence intervals. Data refer to the year 2019 for which both telomere length and PFAS (n=11) and BMI and PFAS data (n=12) were available in this species.

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