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1 **High levels of fluoroalkyl substances and potential disruption of thyroid**
2 **hormones in three gull species from South Western France**

3 Sebastiano M^{a*}, Jouanneau W^a, Blévin P^{a,b}, Angelier F^a, Parenteau C^a, Gernigon J^c, Lemesle J^c,
4 Robin F^{c, d}, Pardon P^e, Budzinski H^e, Labadie P^e, Chastel O^a

5

6 a Centre d'Etudes Biologiques de Chizé (CEBC), UMR 7372 CNRS-Univ. La Rochelle, France

7 b Akvaplan-niva AS, Fram Centre, NO-9296 Tromsø, Norway

8 c Réserve Naturelle de Lilleau des Niges, 17880, France

9 d Ligue pour la Protection des Oiseaux (LPO), 17300 Rochefort, France

10 e Univ. Bordeaux, CNRS, EPOC, EPHE, UMR 5805, F-33600 Pessac, France

11

12 *Corresponding author

13

14 **Abstract**

15 Per- and Poly-fluoroalkyl substances (PFAS) raised increasing concerns over the past years due to
16 their persistence and global distribution. Understanding their occurrence in the environment and
17 their disruptive effect on the physiology of humans and wildlife remains a major challenge in
18 ecotoxicological studies. Here, we investigate the occurrence of several carboxylic and sulfonic
19 PFAS in 105 individuals of three seabird species (27 Great black-backed gull *Larus marinus*; 44
20 Lesser black-backed gull *Larus fuscus graellsii*; and 34 European herring gull *Larus argentatus*)
21 from South western France. We further estimated the relationship between plasma
22 concentrations of PFAS and i) the body condition of the birds and ii) plasma concentrations of
23 thyroid hormone triiodothyronine (TT3). We found that great and lesser black-backed gulls from
24 South Western France are exposed to PFAS levels comparable to highly contaminated species
25 from other geographical areas, although major emission sources (i.e. related to industrial
26 activities) are absent in the region. We additionally found that PFAS are negatively associated with
27 the body condition of the birds in two of the studied species, and that these results are sex-
28 dependent. Finally, we found positive associations between exposure to PFAS and TT3 in the great
29 black-backed gull, suggesting a potential disrupting mechanism of PFAS exposure. Although only
30 three years of data have been collected, we investigated PFAS trend over the study period, and
31 found that great black-backed gulls document an increasing trend of plasma PFAS concentration
32 from 2016 to 2018. Because PFAS might have detrimental effects on birds, French seabird
33 populations should be monitored since an increase of PFAS exposure may impact on population
34 viability both in the short- and long-term.

35 Introduction

36 Per- and Poly-fluoroalkyl substances (PFAS) raised increasing concerns over the past years due to
37 their persistence and global distribution. Because of their high thermal and chemical stability,
38 these synthetic substances have found an application in the manufacturing industry, mostly used
39 as surfactants and additives (Buck et al., 2011), and have been widely produced over the past 50
40 years (Wang et al., 2017). Being extremely persistent in the environment, and due to their long-
41 range transport via atmospheric and oceanic currents, they have been detected worldwide (Giesy
42 and Kannan, 2001). Several studies have found PFAS to accumulate into living organisms (including
43 invertebrates, fishes, amphibians, mammals, and birds) and to biomagnify through food webs
44 (Kannan et al., 2005; Kelly et al., 2009; Simonnet-Laprade et al., 2019), and to date, PFAS exposure
45 represents a global threat to human health and wildlife (Sunderland et al., 2019). Documenting
46 their occurrence in the environment and understanding their disruptive effect on the physiology of
47 humans and wildlife remains a major challenge.

48 Seabirds are long-lived apex predators generally exposed to high levels of environmental
49 contaminants (Elliott and Elliott, 2013; Furness and Camphuysen, 1997), thus they prove
50 particularly valuable to investigate PFAS accumulation in marine food webs especially in northern
51 areas. High levels of PFOS have been found in plasma samples of several seabird species from the
52 Arctic including ivory gulls *Pagophila eburnea* (average concentration of 31 ng/g, Lucia et al.,
53 2017); glaucous gulls *Larus hyperboreus* (average concentration of 47 ng/g, Melnes et al., 2017;
54 and of 134 ng/g, Verreault et al., 2005); in black-legged kittiwakes from Svalbard (average
55 concentration of 10.2 ng/g, Tartu et al., 2014); and in European shags *Phalacrocorax aristotelis*
56 from Isle of May in Scotland (average concentration of 251 ng/g in females and 163 ng/g in males,
57 Carravieri et al. 2020). High levels of PFOS were also found in egg samples of the European shag
58 and common eider *Somateria mollissima* from Norway (average concentration of 36.8 ng/g and

59 37.4 ng/g, respectively, Herzke et al., 2009); in whole blood of the endangered lesser black backed
60 gull *Larus fuscus* from Norway (average concentration of 33.5 ng/g, Bustnes et al., 2008a); and in
61 several other seabird species. However, much work has been devoted to seabirds from the Arctic
62 regions (i.e. considered a sink for environmental contaminants; Barrie et al., 1992; Braune et al.,
63 2014; Wong et al., 2018), or in highly contaminated areas (e.g. China; Xie et al., 2013), while fewer
64 studies have focused on areas with not-known sources of PFAS (i.e. Antarctica, Munoz et al.,
65 2017b). In France, most studies examining PFAS occurrence and exposure in aquatic ecosystems
66 focused on water, sediments, invertebrates, and fishes (Couderc et al., 2015; Fernandes et al.,
67 2018; Munoz et al., 2019; Simonnet-Laprade et al., 2019). However, to the extent of our
68 knowledge, no studies have been carried out on top predators including birds in this area, which
69 may be exposed to concentrations of concern. It is therefore crucial to investigate PFAS exposure
70 in French seabirds to document PFAS occurrence in marine biota and to provide early warning of
71 its effects on their health status.

72 Over the past few years, there has been an increased body of evidence showing that PFAS
73 may i) impact on adipogenesis thus with body condition (Tartu et al., 2014), and ii) disrupt several
74 physiological traits of seabirds. For instance, previous work found that PFAS exposure is associated
75 with lower levels of the stress hormone corticosterone (Tartu et al., 2014), higher oxidative stress
76 (Costantini et al., 2019), longer telomeres (Blévin et al., 2017a; Sebastiano et al., 2020), and a
77 higher metabolic rate (Blévin et al., 2017b). Further studies found PFAS to be associated with
78 higher levels of the parental hormone prolactin and altered incubation behaviours (Blévin et al.,
79 2020), lower hatching success (Tartu et al., 2014), and a higher survival rate (Sebastiano et al.,
80 2020). Specifically, one way through which PFAS may impact on organism function is by disrupting
81 hormonal mechanisms. Previous work provided evidence that PFAS have a strong affinity for
82 proteins and are known to bind to the thyroid hormone transport protein transthyretin (Ren et al.,

83 2016; Weiss et al., 2009). In birds, the hypothalamic-pituitary-thyroid (HPT) axis controls the
84 secretion of the thyroid hormone thyroxine (T4), which is then converted to triiodothyronine (T3),
85 the active form of T4 (McNabb, 2007). Although Blévin et al. (2017b) found no association
86 between PFAS exposure and thyroid hormones in adult black-legged kittiwakes *Rissa tridactyla*,
87 Braune et al. (2011) found a significant positive correlation between total triiodothyronine (TT3)
88 levels and hepatic concentrations of PFAS in northern fulmars. Nøst et al. (2012) also found a
89 positive association between PFAS levels and total thyroxine (TT4) in black-legged kittiwake and
90 northern fulmar chicks, suggesting that PFAS may potentially act through an endocrine disrupting
91 mechanism. More recently, Melnes et al. (2017) found that PFAS were positively associated with
92 free triiodothyronine (FT3) in the glaucous gull. To date, further work is needed to understand the
93 relationship between PFAS exposure and thyroid functioning in birds, especially considering that in
94 birds, T3 and T4 are involved in a multitude of physiological pathways (McNabb, 2007). A
95 disruption of thyroid hormone levels may be detrimental to development, behaviour, and
96 reproduction (McNabb, 2007).

97 The Lilleau des Niges Natural Reserve is an important site for breeding, wintering, and
98 migration of several bird species. It is located north of Ile de Ré, an island off the west coast of
99 France, in front of La Rochelle, in the Bay of Biscay. By hosting several seabird species during the
100 breeding season, this island offers a unique opportunity to investigate the occurrence of PFAS in a
101 French seabird community. Although most previous studies have been carried out on a single
102 species (Blévin et al., 2017b; Costantini et al., 2019; Melnes et al., 2017; Tartu et al., 2014),
103 investigating several species simultaneously and from the same geographical area can help to
104 better understand the mechanisms of exposure to PFAS and the potential physiological
105 consequences of PFAS contamination. For instance, the Herring gull *Larus argentatus*, the lesser
106 black-backed gull *Larus fuscus graellsii*, and the great black-backed gull *Larus marinus*, which

107 breed sympatrically on the island, are characterized by different foraging and migratory strategies,
108 thus potentially exposed to different concentrations of PFAS. The aims of this study were to i)
109 assess to which extent French seabirds are contaminated by PFAS; ii) investigate the relationship
110 between exposure to PFAS and body condition; and iii) determine the association between
111 exposure to PFAS and plasma thyroid hormone T3 concentration in the three above mentioned
112 seabirds from Ile de Ré. Data on PFAS occurrence and their potential adverse effects in seabirds
113 from France are not yet available. To date, we are not aware of known point sources of PFAS in
114 the region. But considering that diverse important rivers may discharge PFAS near the study area
115 (Simonnet-Laprade et al., 2019), and that PFAS may reach and accumulate in remote areas due to
116 their long-range oceanic and atmospheric transport (Munoz et al., 2019), we expect comparable
117 PFAS concentrations with seabirds from the Arctic. In addition, if PFAS have a stimulating effect on
118 thyroid hormone production as found in previous work (DeWitt, 2015; Liu et al., 2011; Nøst et al.,
119 2012), we expect a positive association between PFAS and the concentration of thyroid hormones.
120 Furthermore, although some PFAS are listed as POPs by the Stockholm Convention and their
121 production has subsequently been reduced over the past years, studies investigating temporal
122 trends of PFAS in tissues of wildlife are limited. Although our data have been solely collected over
123 three years of study, we further aim to describe the temporal variation in blood concentration of
124 PFAS from 2016 to 2018 in local seabirds.

125 **Materials and Methods**

126 **Sampling**

127 Field work was performed in 2016, 2017, and 2018 at the Lilleau des Niges Natural Reserve (46°
128 13' 53" N, -1° 30' 22" W), managed by the Ligue pour la Protection des Oiseaux (LPO) located on
129 the North side of Ile de Ré, France, as a part of a monitoring program for PFAS in the region. A
130 total of 108 breeding adult birds from three species were captured during the incubation stage on
131 their nests using a nest trap. Because out of the 108 observations, three were coming from the
132 same individuals sampled at different years, one or the other observation was randomly excluded
133 to perform statistical analyses. Therefore, the final dataset included a total of 105 birds (European
134 herring gull, n=9 in 2016, n=16 in 2017, and n=9 in 2018; lesser black-backed gull, n=11 in 2016,
135 n=17 in 2017, and n=16 in 2018; great black-backed gull, n=9 in 2016, n=7 in 2017, and n=11 in
136 2018). After capture, 2mL of blood was collected from the alar vein using a heparinized syringe
137 and a 25 gauge needle. Blood was kept in a cold container and centrifuged for 10 min at 8,000 x *g*
138 at 20 °C at the laboratory within a few hours after collection; plasma and red blood cells were kept
139 frozen at -20 °C until laboratory analyses. Skull and tarsus were measured with an accuracy of 0.1
140 mm using a caliper. Wing length was also measured with an accuracy of 1 mm using a ruler, and
141 birds were weighted to the nearest 5 g using a Pesola spring balance. Birds were sexed from red
142 blood cells by polymerase chain reaction amplification (PCR) of part of two highly conserved genes
143 (CHD) of sexual chromosomes. Briefly, DNA was extracted from erythrocytes and the sex was
144 determined by molecular sexing based on polymerase chain reaction (PCR) amplification of the
145 CHD gene as described in Fridolfsson and Ellegren (1999). Amplification was performed in 20µl
146 final volume with a Eppendorf Mastercycler using 0.5 U Taq DNA polymerase, 200µM dNTPs,
147 10mM Tris-HCl pH 8.3, 50mM KCl, 1.5mM MgCl₂ and 0.4µM of primers 2550F (5'-
148 GTTACTGATTCGTCTACGAGA-3') and 2718R (5'-ATTGAAATGATCCAGTGCTTG-3'). Female birds may

149 deposit a significant amount of PFAS into their eggs. Therefore, to minimize the variation due to
150 PFAS deposition in eggs, we have only sampled individuals having either two (17/105, 16% of
151 birds) or three eggs (87/105, 83% of birds) except one sampled females that only laid one egg
152 (1/105, 1%). Preliminary statistical analyses were carried out to test whether females with two
153 (8/52, 15% of females) or three eggs (43/52, 83%) had different concentrations of PFAS. However,
154 linear models showed that for any PFAS, concentrations were similar between females that laid
155 two or three eggs (all $t < 1.14$, all $P > 0.26$), thus clutch size was not further included in the statistical
156 analyses.

157 **PFAS analyses**

158 A total of 14 PFAS were analysed in each plasma sample, including eight carboxylates: branched-
159 (Br-PFOA) and linear-perfluorooctanoate (L-PFOA), perfluorononanoate (PFNA),
160 perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorododecanoate (PFDoDA),
161 perfluorotridecanoate (PFTTrDA), perfluorotetradecanoate (PFTeDA); and six sulfonates:
162 perfluorohexanesulfonate (PFHxS), branched- (Br-PFHpS) and linear-perfluoroheptasulfonate (L-
163 PFHpS), branched perfluorooctanesulfonate (Br-PFOS), linear perfluorooctanesulfonate (L-PFOS), and
164 perfluorooctanesulfonamide (FOSA). Analytical standards of native PFAS along with a series of 10
165 ^{13}C , ^{18}O or D mass-labelled internal standards used for quantification purposes were supplied by
166 Wellington laboratories. All reagents were analytical grade or equivalent (see Munoz et al. (2017b)
167 for full details). Briefly, in a 2 mL polypropylene Eppendorf tubes, a 25 μL aliquot of plasma was
168 weighed (~ 25 mg) and internal standards (ISs) were subsequently added under gravimetric
169 control (~ 15 mg of a 1 $\text{pg}/\mu\text{L}$ IS mixture prepared in methanol). Following protein precipitation
170 with 100 μL of acetonitrile (ACN), extracts were centrifuged for 10 min at 24,000 $\times g$ at 20 $^{\circ}\text{C}$. The
171 supernatant was then transferred to 2 mL polypropylene centrifuge tubes (0.22 μm nylon filter).
172 After centrifugation for 3 min at 7,000 $\times g$ at 20 $^{\circ}\text{C}$, extracts were transferred to 2 mL auto sampler

173 glass vials and diluted with 675 μ L of HPLC-water. Extracts were briefly vortexed and then
174 processed using an Agilent Technologies (Massy, France) on-line SPE platform which comprises a
175 standard auto sampler (1260 Infinity ALS), a quaternary pump (1260 Infinity Quaternary Pump VL),
176 a switch valve (1200 2 Position/6 Port Valve) and an on-line SPE column support (1200 6 Position
177 Selection Valve), which were all automatically controlled via the Acquisition module of the Agilent
178 Mass Hunter software as previously done (Munoz et al., 2017b). HPLC-water aliquots were run
179 between each seabird plasma sample to eliminate any cross-contamination. Note that on-line
180 extraction was performed with Waters Oasis HLB on-Line SPE columns (2×10 mm, dp= 25-35 μ m)
181 while analyte separation was carried out using an Agilent C₁₈ Poroshell analytical column ($2.1 \times$
182 100 mm, 2.7 μ m).

183 **Quality assurance/Quality control (QA/QC)**

184 When analytes were detected in blanks, blank correction was performed and a limit of reporting
185 (LOR) was defined as three times the maximum blank signal divided by the average mass of
186 plasma used for analysis. A limit of detection (LOD) was also defined as the concentration yielding
187 a signal to noise ratio of 3 in spiked plasma samples. Because laboratory analyses were performed
188 in different years, a unique left-censoring threshold was set for each analyte, i.e. the maximum
189 between LORs and LODs, all years combined. For those PFAS with concentrations below this
190 threshold in less than 30% of samples, left-censored data were arbitrarily replaced with $\frac{1}{2} \times$ LOR or
191 LOD to enable statistical analyses. Therefore, ten PFAS (PFNA, PFDA, PFUnDA, PFDoDA, PFTTrDA,
192 PFTeDA, PFHxS, L-PFHpS, Br-PFOS, and L-PFOS) could be further investigated (i.e. other analytes
193 were excluded from statistical analyses). LORs, LODs and detection frequencies are presented in
194 the supplementary information (Table S1). For each sample batch (20 samples), several QA/QC
195 points were assessed by analyzing: i) two procedural blanks consisting of 25 μ L of HPLC-water that
196 went through the entire analytical procedure; ii) one human serum standard reference material

197 (NIST SRM 1957, trueness assessment); iii) replicate spiked chicken plasma samples (target
198 analytes added jointly with mass-labelled ISs at the beginning of the preparation procedure at 2
199 ng/g each, accuracy assessment); and iv) HPLC-water samples spiked at 2 ng/g, accuracy
200 assessment) as previously described (Munoz et al. 2017b). Procedural blanks showed very limited
201 contamination. The analysis of NIST SRM 1957 gave satisfactory results, i.e. within the specified
202 uncertainty interval. For those compounds with a reference concentration (i.e. PFHpA, PFOA,
203 PFNA, PFDA, PFUnDA, PFHxS and PFOS), levels deviated between 2 and 20% from the reference
204 concentration (except for FOSA, which deviated 36%).

205 **Thyroid hormone analyses**

206 TT3 was determined by radioimmunoassay. Briefly, 25 µL of plasma was incubated for 24h at 4 °C
207 with a known concentration (10000 cpm) of T3 marked with the radioisotope Iodine-125 (T3-¹²⁵I,
208 Perkin Elmer, US, reference: NEX110X100UC) and an antibody *Ab* (polyclonal rabbit antiserum,
209 Sigma-Aldrich, US, reference: T-2777). Because *Ab* is available in a limited concentration, T3 and
210 T3-¹²⁵I compete for *Ab*, to which they bind. Therefore, after incubation, there is a bound fraction
211 (T3 and T3-¹²⁵I bound to *Ab*) and a free fraction (T3 and T3-¹²⁵I unbound to *Ab*), which are
212 separated by adding a sheep anti-rabbit antibody (whole anti-serum anti rabbit IgG produced in
213 sheep), incubated for 12h at 4 °C followed by centrifugation at 4,300 x *g* at 18-20°C for 45 min. The
214 bound fraction is then counted with a wizard 2 gamma counter (Perkin Elmer, US). Pooled plasma
215 of diverse gull samples were serially diluted and produced a dose-response curve parallel to the T3
216 standard curve. The lowest TT3 detectable concentration was 0.07 ng/ml (LOD). Samples below
217 this limit (n=3) were replaced with a value equal to ½ x LOD to enable statistical analyses. All
218 samples were run in duplicates. Samples that had a coefficient of variation above 15% and could
219 not be done in triplicates due to low plasma volume were not included in statistical analyses
220 (n=11). An additional measurement of TT3 was excluded from statistical analyses since it was

221 considered an outlier (the measurement exceeded the mean \pm 3 times the standard deviation and
222 was highly influential in statistical analyses). Therefore, for a total of 58 samples (n=6 in 2016 and
223 n=12 in 2017 for the European herring gull; n=10 in 2016 and n=14 in 2017 in lesser black-backed
224 gull; n=8 in 2016 and n=8 in 2017 in great black-backed gull), both TT3 and PFAS data were
225 available. The intra-assay coefficient of variation was 9.73%, while the inter-assay coefficient of
226 variation amounted to 15.13%.

227 **Statistical analyses**

228 After PFAS data were log-transformed to reduce the influence of extreme values (see below),
229 linear models were used to investigate differences in PFAS concentrations among species and
230 between genders (in samples collected from 2016 to 2018). In each model, each PFAS was
231 considered as a dependent variable while the factors *Species*, *Sex*, and their interaction were
232 considered as predictors. To simultaneously investigate species specific temporal trends in PFAS
233 exposure from 2016 to 2018, the *Year*, the factor *Species*, and their interaction were also included
234 in the model as explanatory variables. We used a similar model to test the difference in body
235 condition between sexes. Briefly, the body condition has been calculated using the body mass
236 adjusted by a linear body measurement (i.e. skull length) using the formula described in Peig and
237 Green (2009).

238 Linear models were additionally used to study the association between TT3 and PFAS (in samples
239 collected in 2016 and 2017). In these models, a three-way interaction between *PFAS*, the factors
240 *Species* and *Sex*, was used to investigate sex-related responses to PFAS exposure. These models
241 additionally included the *Year* (as a factor, to control for the temporal variation in TT3 and PFAS),
242 and *body condition* (as a covariate, to control for the individual condition of birds). A similar model

243 was built to test the association between the body condition and PFAS in all samples collected
244 from 2016 to 2018.

245 All PFAS concentrations (except PFTeDA, which was normally distributed and assumptions listed
246 below were respected without data transformation), were log-transformed when testing for time
247 trends and when testing for inter-species and between sex differences. All PFAS concentrations
248 were log-transformed when testing for the association between either TT3 or body condition and
249 PFAS. Data transformation was done to meet model assumptions as homoscedasticity and
250 normality of residuals, further confirmed by visually inspecting Q-Q plots. All data transformation
251 and violation of models' assumptions are reported throughout the manuscript. Statistical
252 significance was set to $\alpha=0.05$ and 95% confidence intervals were used during data processing and
253 data visualization. All statistical analyses were performed using R version 3.5.2.

254

255 **Results**

256 PFAS used in statistical analyses were detected in all samples (Table S1) and their concentrations
257 are summarized in Table 1 and Figure 1. PFOS was the most abundant, followed by the odd-chain
258 carboxylates PFTrDA and PFUnDA. Linear models showed statistically significant differences
259 among species for all carboxylic and sulfonic PFAS (all $F > 5.15$, all $P < 0.01$), and all statistical outputs
260 and post-hoc differences can be found in Table S2. Among carboxylates, PFNA levels were higher
261 in herring gulls and lesser black-backed gulls than great black-backed gulls (both $P < 0.05$, Figure 1),
262 PFDA levels were higher in great black-backed gulls than herring gulls ($P < 0.01$, Figure 1), PFUnA,
263 PFDoDA, PFTrDA, and PFTeDA levels were higher in great and lesser black-backed gulls than
264 herring gulls (all $P < 0.01$, Figure 1), and PFTrDA levels were also higher in great than lesser black-
265 backed gulls ($P < 0.05$, Figure 1). Among sulfonic acids, PFHxS was higher in both lesser black-
266 backed and herring gulls than great black-backed gulls (both $P < 0.01$, Figure 1), PFHpS and L-PFOS
267 were highest in lesser black-backed gulls (all $P < 0.001$, Figure 1), and Br-PFOS was higher in lesser
268 than great black-backed gulls ($P < 0.05$, Figure 1).

269 All carboxylates showed significantly higher concentrations in males than in females for all species
270 (all $P < 0.05$, except for PFNA in the herring gull, for which $P = 0.07$; Table 1). Among sulfonates,
271 PFHxS showed similar concentrations between females and males in all species (all $P > 0.16$, Table
272 1), while PFHpS, Br- and L-PFOS showed significantly higher concentrations in males than females
273 (all $P < 0.05$, Table 1). In all three species, there was no difference in the body condition between
274 males and females (all $t < 1.27$, all $P > 0.80$). Finally, TT3 levels were similar between sexes in all
275 three species ($t = 2.76$, $P = 0.08$).

276 In great black backed gulls, TT3 was positively associated with PFUnDA, PFDoDA, PFTrDA, PFTeDA
277 and Br-PFOS in females (all $t > 2.10$, all $P \leq 0.04$; Figure 2a-d, 2f, Table S3), while TT3 was negatively

278 associated with PFHxS in males ($t=-2.69$, $P=0.01$; Figure 2e, Table S3). There was no association
279 between TT3 and any PFAS in herring gulls and lesser black-backed gulls (all $t\leq 0.79$, all $P\geq 0.43$,
280 Table S3).

281 In great black backed gull females, increasing levels of PFNA, PFDA, PFHxS, PFHpS, Br- and L-PFOS
282 were associated with a reduced body condition (all $t\leq -2.19$, all $p\leq 0.03$; Figure S1, S2, Table S4),
283 while increasing levels of PFNA and PFDA were associated with a reduced body condition (both $t\leq$ -
284 2.18, both $p=0.03$; Figure S1, Table S4) in lesser black-backed gull males, but not females.

285 In great black backed gulls, there was a significant or marginally-significant increase in all PFAS
286 from 2016 to 2018 (all $t\geq 1.93$ and all $P\leq 0.056$; Figure 3 and 4, Table S5), while lesser black backed
287 gulls showed an increase in PFTeDA and PFHxS, and a marginally significant decrease in PFUnDA
288 levels from 2016 to 2018 (all $t\geq 1.98$, all $P\leq 0.05$; Figure 3, 4, Table S5). All PFAS showed similar
289 concentrations among years in the herring gulls except for PFTTrDA, which increased from 2016 to
290 2018 ($t=2.30$, $P=0.02$; Figure 3, Table S5). Body condition did not change in any of the species from
291 one year to another (all $t< 1.14$, all $P> 0.26$). TT3 levels remained similar between 2016 and 2017 in
292 all three species (all $t< 1.88$, all $P> 0.42$).

293

294 **Discussion**

295 Our study is the first to provide evidence that although not-known point sources of emission are
296 present in the region, several PFAS were detected in seabird species from South Western France.
297 Great and lesser black-backed gulls show that both plasma carboxylate and sulfonate
298 concentrations are comparable to highly contaminated seabird species from Arctic regions, while
299 herring gulls are exposed to relatively lower levels of PFAS. We found that PFAS are negatively
300 associated with the body condition of the birds. Furthermore, TT3 levels were associated with
301 several PFAS in a contrasted manner between sexes in the great black-backed gull, suggesting a
302 potential disrupting mechanism of PFAS exposure. Finally, the great black-backed gulls
303 documented an increasing trend of plasma PFAS concentration from 2016 to 2018.

304 Our results show that among carboxylates, PUnDA and PTrDA are the most abundant
305 congener in all three species, a pattern that is commonly found in seabird species (Bustnes et al.,
306 2008b; Melnes et al., 2017; Tartu et al., 2014). Because of the strong winds and oceanic currents
307 that characterize the Atlantic Ocean, and considering that, to the best of our knowledge, there are
308 no point sources of PFAS in the region, perfluorinated compounds should occur at a lower
309 concentration than in the Mediterranean and the Arctic regions, considered as sinks for pollutants
310 (Danovaro, 2003; Wong et al., 2018). However, the levels of carboxylates found in this study
311 (ranging from a median of 0.6 ng/g of PTeDA in Herring gulls to 5.8 ng/g of PTrDA in great black-
312 backed gulls) are similar to those reported for glaucous gulls (ranging from a median of 0.1 ng/g of
313 PFOA to 3.8 ng/g of PUnDA, Melnes et al., 2017) and lesser black-backed gulls (ranging from a
314 median of 0.2 ng/g of PDoDA to 5.9 ng/g of PFDA, Bustnes et al., 2008a) from Arctic regions, but
315 lower than those found in other species (e.g. in black-legged kittiwakes from Svalbard; ranging
316 from a mean of 1.0 ng/g of PFNA to 18.2 ng/g of PTrDA, Tartu et al., 2014). One possible
317 explanation may be related to the continental input of PFAS through the Gironde, Loire, and

318 Charente estuaries (Munoz et al., 2019; Munoz et al., 2017a; Munschy et al., 2019), which may
319 have contributed to the observed concentrations. Except for PFNA (which levels were higher in
320 herring gulls and lesser black-backed gulls than great black-backed gulls), most carboxylates were
321 higher in lesser and great black backed gulls than herring gulls. Although we cannot exclude that
322 these three species differ in their ability to excrete PFAS from their body, our results suggest that
323 the differences in exposure likely depend on the trophic niche occupied by the species. For
324 instance, a recent study on PFAS in six seabird species from the Arctic regions showed that
325 predatory birds (e.g. Great skua *Stercorarius skua*, 44.8 ng/g of sumPFAS) showed the highest
326 contaminant load compared with species from a lower trophic level (e.g. Common eider *Somateria*
327 *mollissima*, 1.3 ng/g of sumPFAS; Haarr et al. 2018). Great black-backed gulls feed on higher
328 trophic level preys and mainly forage along the shore (Maynard and Davoren, 2020), while lesser
329 black-backed and herring gulls are known to have a generalist diet which also includes food items
330 from both terrestrial and marine origin (Corman et al., 2016; Maynard and Davoren, 2020).

331 Among sulfonates, L-PFOS was the most abundant, followed by Br-PFOS, PFHxS, and PFHpS
332 and all occurred at very high concentrations. For instance, PFHxS ranged from a median value of
333 1.2 ng/g in great black-backed gulls to 2.2 ng/g in lesser black-backed gulls, while other studies
334 from highly contaminated areas reported lower PFHxS plasma concentrations (a median below 1
335 ng/g in lesser black-backed gulls, Bustnes et al., 2008b; a median below 0.7 in glaucous gulls,
336 Melnes et al., 2017; all samples below 0.2 ng/g in black-legged kittiwakes, Tartu et al., 2014).
337 Furthermore, L-PFOS ranged from a median of 11.6 ng/g in herring gull females to a median of
338 54.7 ng/g in lesser black-backed gull males. Thus, lesser black-backed gulls in our study showed
339 very high sulfonate levels, even higher than Norwegian populations (a median of 40 ng/g of PFOS
340 in males, Bustnes et al., 2008b), and in this species, sulfonate levels are significantly higher than
341 those observed in great black-backed gulls. In addition, plasma concentrations of most PFAS

342 showed significantly higher levels in males than in females, but this difference was not related to
343 the body condition of the birds, with males showing a similar body condition than females.
344 Because females transfer contaminants in the eggs, it is thus possible that females have lower
345 levels of circulating PFAS in plasma. However, previous work pointed out contrasting results
346 between PFAS in eggs and plasma, suggesting that the extent of PFAS transfer to the eggs may
347 significantly vary among the studied species (Bustnes et al., 2008a; Herzke et al., 2009; Verreault
348 et al., 2005). Given that in this study we did not analyse PFAS levels in eggs, it is not possible to
349 clarify whether the species differ in terms of PFAS excreted in eggs. Although Verreault et al.
350 (2006) found that the contaminant content in glaucous gull eggs fluctuated irrespectively of the
351 laying order, other work suggests that the majority of PFAS are found in the first or the first two
352 eggs, while negligible concentrations of PFAS are found in the third egg, as previously shown in
353 Audouin's gulls *Larus audouinii* (Vicente et al., 2015). Our results showed that females that laid
354 three eggs had similar PFAS concentrations than females that laid two eggs, therefore our results
355 should not be affected by the difference in PFAS deposition in eggs.

356 Because of the higher bioaccumulative properties and biomagnification and of longer-chain
357 PFAS (Boisvert et al., 2019; Simonnet-Laprade et al., 2019), these compounds tend to occur at
358 higher concentrations in wildlife tissues (Conder et al., 2008; Muir et al., 2019; Muir and de Wit,
359 2010). Therefore, individuals feeding at a higher trophic position are likely to be exposed to higher
360 concentrations of long-chained carboxylates. Previous work also showed that longer chained PFAS
361 are more likely to induce adverse health effects in seabirds compared to shorter chained PFAS. For
362 instance, negative associations between PFAS and baseline corticosterone in black-legged
363 kittiwakes were only found for PFTTrDA and PFTTeDA (Tartu et al., 2014), while such association was
364 not found for shorter-chain PFAS. Additional work on the same bird population found higher
365 protein oxidative damage in those birds having higher concentrations of PFDoDA, PFTTrDA and

366 PFTeDA (Costantini et al., 2019). Similarly, previous work found a positive association between
367 PFTrDA and metabolic rate in the same species (Blévin et al., 2017b). These results were further
368 corroborated by experimental work on rat *Rattus sp.* cell cultures, showing that the cytotoxicity of
369 PFAS increases with increasing carbon chain length (Berntsen et al., 2017), and that comparing
370 molecules with a similar chain length, a sulfonate functional group may lead to greater toxicity
371 than a carboxyl group (Berntsen et al., 2017).

372 Our results are thus of particular interest as they suggest that birds feeding at a higher
373 trophic position and showing a more marine diet (i.e. great black-backed gulls) should also be
374 exposed to greater toxicological risks from carboxylates, while birds with a more generalist diet
375 (i.e. lesser black-backed gulls) can be exposed to higher levels of sulfonates. This is likely the
376 reason why we found associations between TT3 and PFAS only in great black-backed gulls, while
377 no significant associations have been found in herring gulls and lesser black-backed gulls.
378 Interestingly, our results were dependent on the sex of the birds. Indeed, although we found that
379 PFHxS was associated with TT3 in great black-backed gull males, all other associations were found
380 in females only. Despite the lower concentration of circulating plasma PFAS levels, females can
381 deposit PFAS in eggs therefore we cannot be certain that females were exposed to lower PFAS
382 concentrations than males, and further work including other tissues (e.g. liver or muscle) would
383 clarify whether PFAS intake differs between the sexes of sampled birds. Despite the absolute
384 concentrations to which they are exposed, a possible explanation for the results in females may
385 rely on the fact that incubation can be extremely costly for female birds (Hanssen et al., 2005),
386 thus they may be more susceptible to PFAS exposure. Previous work in birds suggest a modulation
387 of thyroid function induced by exposure to various environmental contaminants. Smits et al.
388 (2002) reported decreased TT3 levels in American kestrels *Falco sparverius* experimentally
389 exposed to PCBs, while Verreault et al. (2004) reported a decrease in T4:T3 ratio in the glaucous

390 gull. Similarly, exposure to organochlorines was associated with reduced TT3 in kittiwakes (Blévin
391 et al., 2017b), and with reduced T3 and T4 in glaucous gulls (Melnes et al., 2017; Verreault et al.,
392 2004). However, specifically related to PFAS, further work on seabirds found a positive association
393 with thyroid functioning (i.e. between PFOS and FT3 in glaucous gulls, Melnes et al., 2017;
394 between several PFAS and TT4 in black-legged kittiwakes and northern fulmars, Nøst et al., 2012).
395 Although being conducted in fish, an experimental approach showed that exposure to PFOS in
396 zebra fish (*Danio rerio*) led to increased thyroid hormones secretion (Liu et al., 2011). Thus, our
397 results on great black backed gulls are in line with previous studies. In this study, increasing TT3
398 levels in this species were found with increasing concentrations of longer chain PFAS (PFUnDA,
399 PFDoDA, PFTTrDA, and PFTeDA) and Br-PFOS. This suggests that despite carboxylates and
400 sulfonates are functionally different, their effect on TT3 is similar. However, this was not the case
401 for PFHxS, which showed a decrease in TT3 levels with increasing concentrations. PFHxS is highly
402 toxic and causes thyroid disruption by lowering thyroid hormone levels in rats (Ramhøj et al.,
403 2020), but it remains unclear why this effect was only found in great black-backed gull males.
404 Indeed, females showed similar levels than males, and the other species exhibited higher PFHxS
405 concentrations than those found in great black backed gulls, thus this result would strongly benefit
406 from experimental support. Because the avian thyroid gland secretes almost exclusively T4 (Darras
407 et al., 2006), most T3 is derived from the deiodination of T4 (Darras et al., 2006). A possible
408 explanation is that in great black-backed gulls, exposure to PFHxS may negatively impact either the
409 transport of T4 (by reducing the activity of serum binding proteins) or deiodination processes. It is
410 therefore strongly warranted to supplement in vitro experiments to verify the effect of PFHxS on
411 T4 transformation. Our results do not provide evidence for a causal relationship PFAS exposure
412 and circulating thyroid hormones. But the contrasting results found between sexes strongly call for

413 further work to experimentally investigate the effect of PFAS exposure on thyroid functioning of
414 birds.

415 Sex-related differences were also found while investigating the relationship between
416 exposure to PFAS and the birds' body condition. In female great black-backed gulls, we found that
417 increasing concentrations of PFNA, PFDA, and all four sulfonates were negatively associated with
418 body condition, while in lesser black-backed gulls, a similar negative relationship between PFNA,
419 PFDA, and body condition was only found in males. Previous work in humans and various animal
420 models found that exposure to certain PFAS is suspected to disrupt fatty acids metabolism and
421 promote adipogenesis (Cheng et al., 2016; Wan et al., 2012; Xu et al., 2016; Yeung et al., 2007).
422 More specifically, these changes in lipid content are related to the capacity of PFAS to alter the
423 expression of genes involved in the metabolism of lipids and fatty acids (Jacobsen et al., 2018;
424 Wan et al., 2012). To date, work on the effect of PFAS on lipid metabolism and body condition in
425 birds remain extremely limited. The negative association we found is in contrast with a previous
426 study on black-legged kittiwakes, showing that PFNA was positively associated with body condition
427 in males (Tartu et al., 2014). One possible explanation for the negative relationship found in
428 females may be related to the ability to deposit PFAS into the eggs. For instance, great black-
429 backed gull females in a better body condition may be more efficient in eliminating PFAS through
430 egg-deposition, although evidences to support this statement are lacking. However, this would not
431 explain why in a closely related species (i.e. lesser black-backed gull) a similar association has been
432 found in males. Our study results are novel but emphasize the need to experimentally investigate
433 the potential association between exposure to PFAS and body condition in birds.

434 Finally, not only does our work provide evidence of high PFAS levels in seabirds from
435 metropolitan France, but our results clearly suggest increasing blood concentration of most PFAS
436 over a relatively short period of time (i.e. from 2016 to 2018) in great and lesser black-backed

437 gulls. A previous study showed that birds caught later on over the breeding season had lower
438 concentration of PFAS (Bustnes et al., 2008a). However, all birds included in this study were
439 sampled during the same period of the year (difference of a few days from one year to another),
440 and, more importantly, all birds were sampled while incubating eggs, thus during the same
441 reproductive state, which should not affect the results. Additionally, we cannot exclude that we
442 unintentionally captured older birds in more recent years (assuming that PFAS levels increase with
443 age in these species). However, considering that all individuals included in this study were adults,
444 and assuming that PFAS concentrations in birds reach a steady level relatively early in life as
445 previously shown for organochlorines (Bustnes et al., 2003), these trends should not be affected
446 by the age of the bird. Information on temporal trends of PFAS in birds' tissues in recent years are
447 scarce and do not exhibit any overall trend (Jouanneau et al., 2020; Land et al., 2018; Muir et al.,
448 2019; Sun et al., 2019). Thus, although only three years of data could be included in the present
449 study, our results provide valuable information on PFAS trends in South western France. Further
450 work including several years of study is strongly warranted to corroborate these findings.

451

452 **Conclusions**

453 Our study provides the first evidence of the presence of high levels of PFAS in seabirds from South
454 western France. Despite some PFAS showed similar levels of other seabird species, L-PFOS and
455 some other PFAS showed either comparable or higher levels than highly contaminated seabird
456 species, and may therefore pose a threat to long-lived seabirds. This hypothesis is further
457 corroborated by our results showing an association between PFAS and the level of the thyroid
458 hormone TT3. Similarly, we provide evidence that PFAS may interfere with lipid accumulation and
459 body condition in birds, and we call for further work to experimentally test this hypothesis. Work

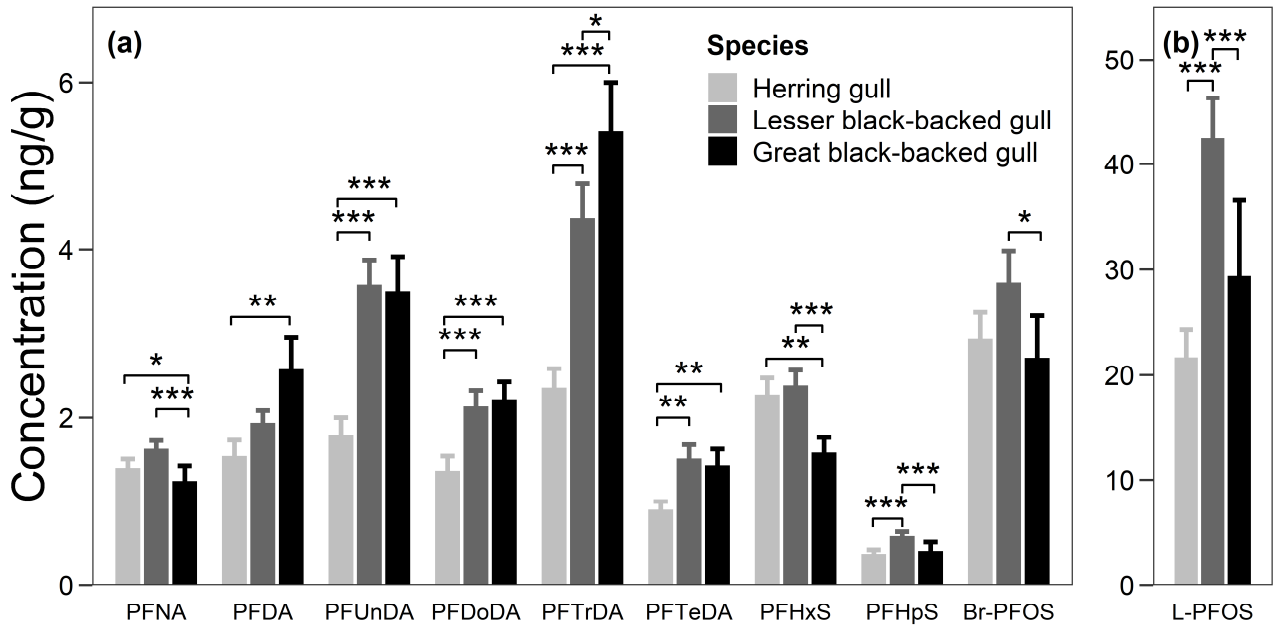
460 on species-specific mechanisms of contaminant excretion and susceptibility to PFAS exposure
461 would prove useful to understand the consequences of PFAS exposure in different species. Our
462 results also document an increase in blood PFAS concentrations over time, particularly in great
463 black-backed gulls, suggesting that PFAS concentrations may also be increasing in the investigated
464 species. Because PFAS have detrimental effects on birds, these and other seabird populations
465 should be monitored as an increase of PFAS exposure may impact on population viability both in
466 the short- and long-term.

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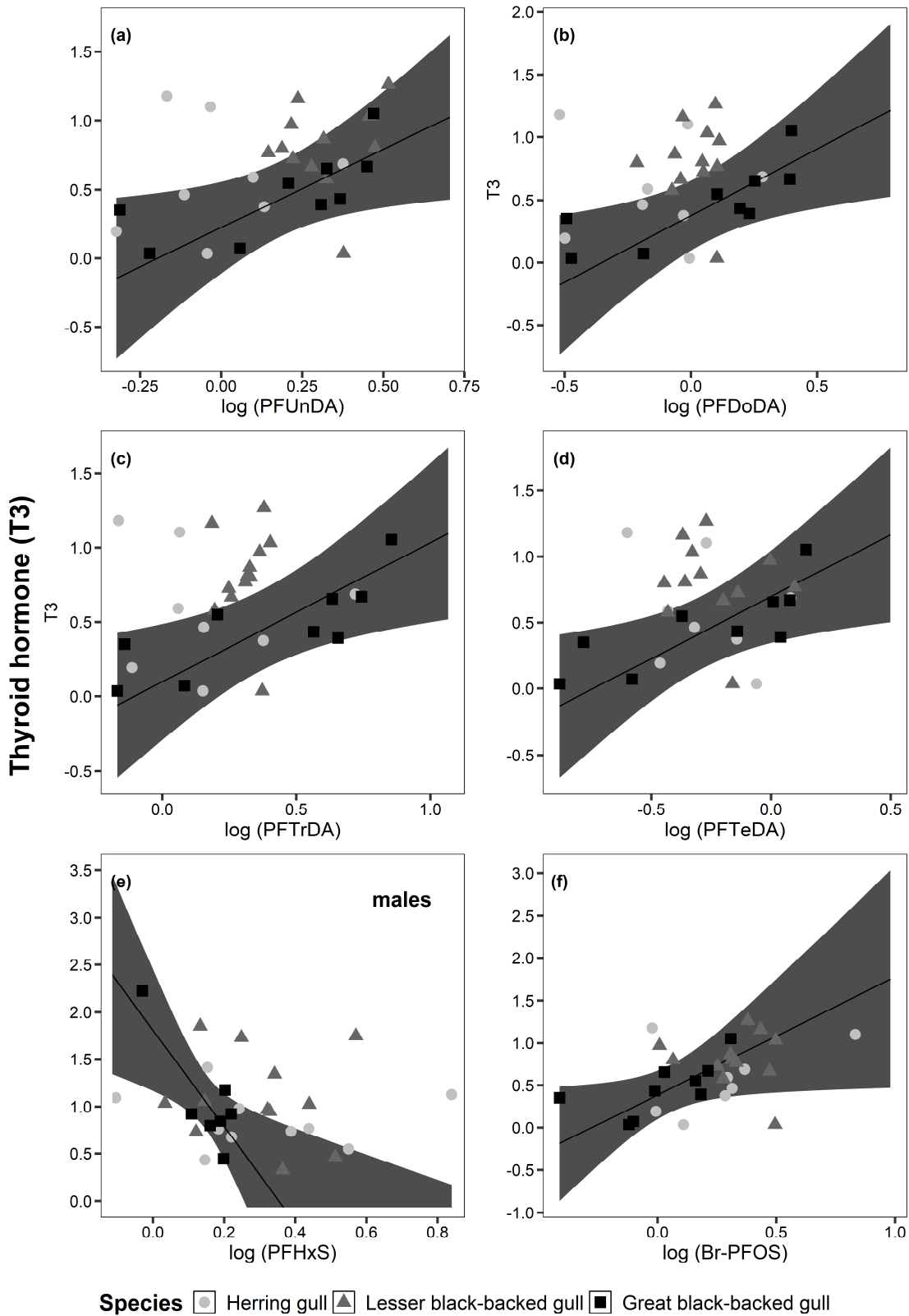
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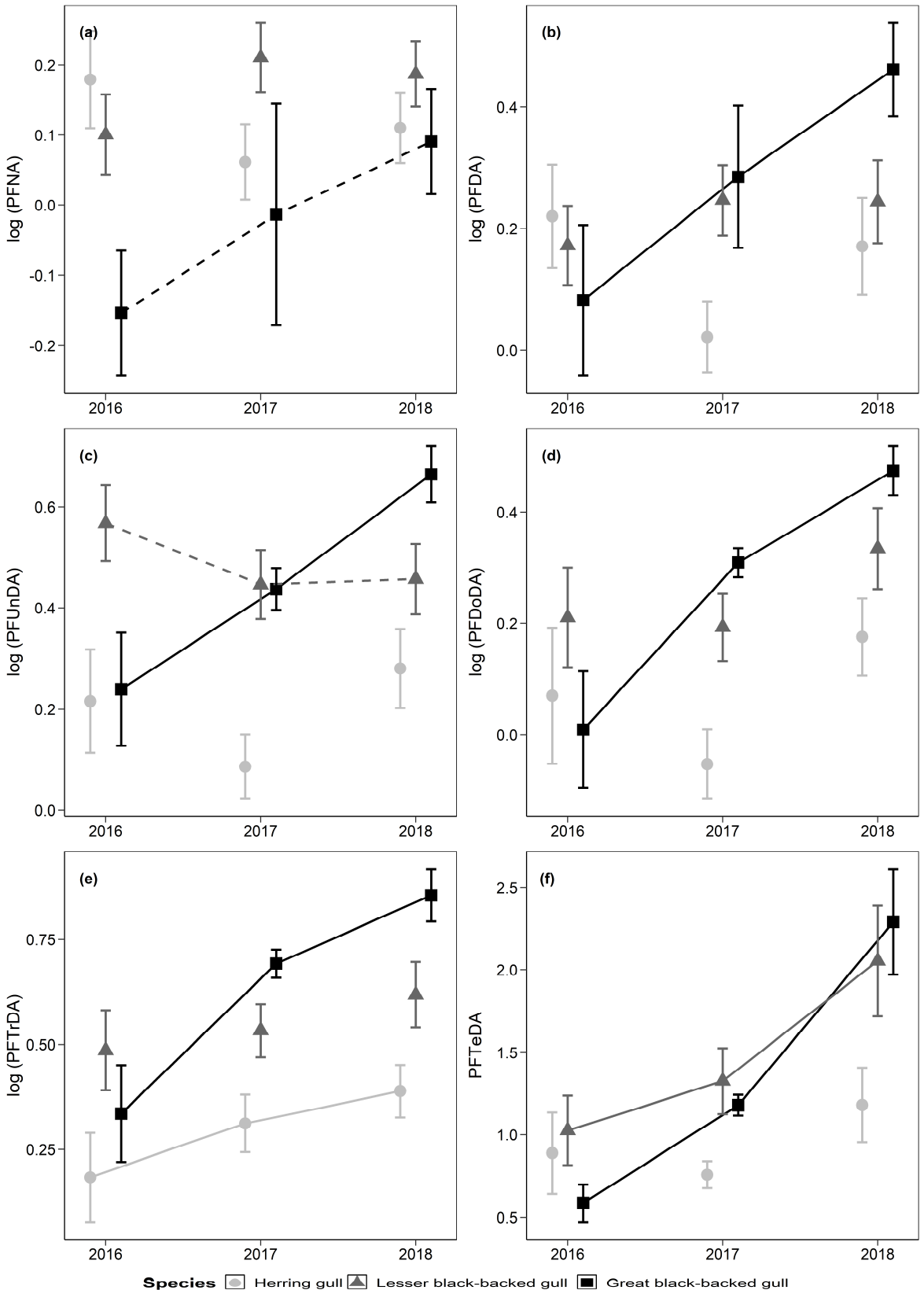
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Figure 1: Plasma concentrations of PFAS (expressed as ng/g of ww) in the three seabird species from Ile de Re. Statistically significant differences are indicated by the asterisk; *, **, ***, indicate a P-value <0.05, <0.01, and <0.001, respectively.



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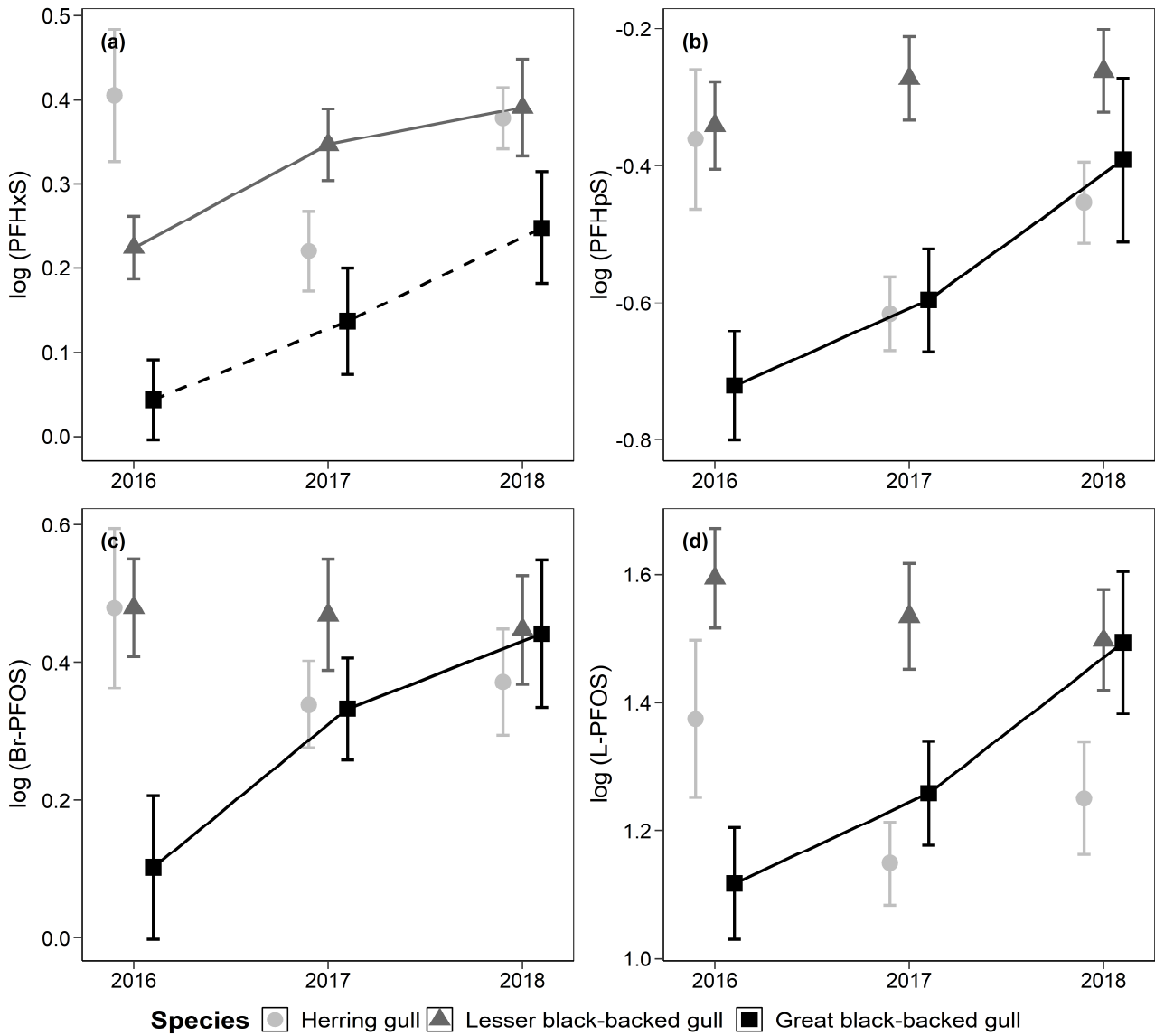
485 **Figure 2:** Relationship between the concentration of the thyroid hormone TT3 (expressed as ng/mL) and
 486 log-transformed carboxylic (C-11 to C-14, panel a to d, respectively), and sulfonic PFAS (PFHxS and Br-PFOS,
 487 panel e and f, respectively) of the three seabird species from Ile de Re. The solid line represents the trend
 488 while the grey area represents 95% confidence intervals. Shapes of data points and three different grades
 489 of grey are used to distinguish the three species as explained in the figure legend. Only significant trends
 490 are shown. Data refer to the period 2016-2017 for which both TT3 and PFAS were available (n=58).



491

492 **Figure 3:** Error bar plots mean ± standard error of carboxylate concentrations in 2016, 2017, and 2018 in
 493 the seabird species from Ile de Re. The shapes and the three different grades of grey are used to distinguish
 494 the three species as explained in the figure legend. Only significant trends are shown. Dashed lines indicate
 495 a trend close to significance.

496



498

499 **Figure 4:** Error bar plots mean \pm standard error of sulfonate concentrations in 2016, 2017, and 2018 in the
 500 seabird species from Ile de Re. The shapes and the three different grades of grey are used to distinguish the
 501 three species as explained in the figure legend. Only significant trends are shown. Dashed lines indicate a
 502 trend close to significance.

503

504 **Table 1:** PFAS concentration (ng/g of ww) in females and males of the three seabird species from Ile de Re.
 505 P-values refer to the difference in PFAS between females and males. Significant P-values are bolded. SE
 506 refers to standard errors.

Herring gull <i>Larus argentatus</i>					
	Females (n=18)		Males (n=16)		P-value
	mean ± SE	median (range)	mean ± SE	median (range)	
PFNA	1.16 ± 0.11	1.06 (0.36, 2.16)	1.68 ± 0.17	1.63 (0.67, 2.85)	0.07
PFDA	1.16 ± 0.19	0.94 (0.38, 4.01)	1.98 ± 0.30	1.79 (0.69, 6.04)	0.01
PFUnDA	1.23 ± 0.17	1.01 (0.47, 3.45)	2.43 ± 0.34	2.17 (0.61, 6.49)	<0.001
PFDoDA	0.95 ± 0.16	0.9 (0.3, 3.22)	1.82 ± 0.32	1.56 (0.55, 6.17)	<0.001
PFTTrDA	1.76 ± 0.27	1.42 (0.55, 5.25)	3.03 ± 0.29	3.05 (1.04, 4.81)	<0.001
PFTeDA	0.61 ± 0.07	0.61 (0.25, 1.23)	1.23 ± 0.16	1.03 (0.33, 2.8)	0.04
PFHxS	2.26 ± 0.21	2.16 (0.88, 3.5)	2.29 ± 0.36	1.89 (0.79, 6.92)	0.99
PFHpS	0.27 ± 0.02	0.25 (0.11, 0.5)	0.49 ± 0.09	0.38 (0.11, 1.63)	0.045
Br-PFOS	2.03 ± 0.34	1.71 (0.86, 6.8)	3.95 ± 0.45	3.75 (1.07, 7.9)	<0.001
L-PFOS	13.82 ± 2.0	11.64 (5.57, 37.04)	30.44 ± 4.31	28.44 (6.6, 62.52)	<0.001

Lesser black-backed gull <i>Larus fuscus graellsii</i>					
	Females (n=20)		Males (n=24)		P-value
	mean ± SE	median (range)	mean ± SE	median (range)	
PFNA	1.14 ± 0.09	1.11 (0.46, 1.83)	2.04 ± 0.1	2.06 (0.95, 3.36)	<0.001
PFDA	1.1 ± 0.08	1.15 (0.54, 1.64)	2.63 ± 0.15	2.58 (1.17, 4.16)	<0.001
PFUnDA	1.88 ± 0.16	1.69 (0.66, 3.28)	5 ± 0.29	4.89 (1.98, 8.7)	<0.001
PFDoDA	1.02 ± 0.07	1.02 (0.39, 1.87)	3.06 ± 0.2	2.86 (1.75, 5.52)	<0.001
PFTTrDA	1.97 ± 0.12	2.06 (0.66, 2.91)	6.38 ± 0.44	5.74 (3.53, 11.66)	<0.001
PFTeDA	0.66 ± 0.07	0.61 (0.13, 1.39)	2.23 ± 0.2	1.87 (1.1, 4.35)	<0.001
PFHxS	2.18 ± 0.25	1.95 (1.12, 6.01)	2.56 ± 0.26	2.24 (1.08, 5.56)	0.94
PFHpS	0.37 ± 0.03	0.35 (0.16, 0.82)	0.79 ± 0.07	0.69 (0.34, 1.84)	<0.001
Br-PFOS	1.86 ± 0.19	1.96 (0.58, 3.15)	5.06 ± 0.5	4.41 (2.38, 13.67)	<0.001
L-PFOS	21.09 ± 2.06	22.76 (5.74, 34.49)	60.23 ± 4.44	54.68 (26.51, 119.69)	<0.001

Great black-backed gull <i>Larus marinus</i>					
	Females (n=14)		Males (n=13)		P-value
	mean ± SE	median (range)	mean ± SE	median (range)	
PFNA	0.59 ± 0.06	0.59 (0.24, 0.95)	1.94 ± 0.29	1.72 (0.89, 4.85)	<0.001
PFDA	1.41 ± 0.23	1.13 (0.32, 3.55)	3.83 ± 0.58	3.34 (1.52, 7.2)	<0.001
PFUnDA	2.42 ± 0.35	2.23 (0.49, 4.93)	4.67 ± 0.65	4.08 (2.62, 11.46)	0.01
PFDoDA	1.69 ± 0.24	1.63 (0.32, 3.12)	2.79 ± 0.29	2.37 (1.68, 5)	0.04
PFTTrDA	4.04 ± 0.72	3.55 (0.68, 9.61)	6.87 ± 0.83	5.79 (3.85, 14.39)	0.02
PFTeDA	0.96 ± 0.17	0.92 (0.13, 2.4)	1.94 ± 0.32	1.42 (0.91, 4.17)	0.03
PFHxS	1.2 ± 0.11	1.16 (0.62, 2.06)	2.01 ± 0.32	1.58 (0.93, 5.33)	0.16
PFHpS	0.2 ± 0.03	0.15 (0.09, 0.47)	0.63 ± 0.21	0.42 (0.22, 3.01)	<0.001
Br-PFOS	1.32 ± 0.17	1.1 (0.39, 2.53)	4.21 ± 0.89	3.21 (1.85, 13.01)	<0.001
L-PFOS	13.45 ± 1.52	10.53 (5.18, 25.92)	46.55 ± 13.74	27.62 (16.71, 194.72)	<0.001

507

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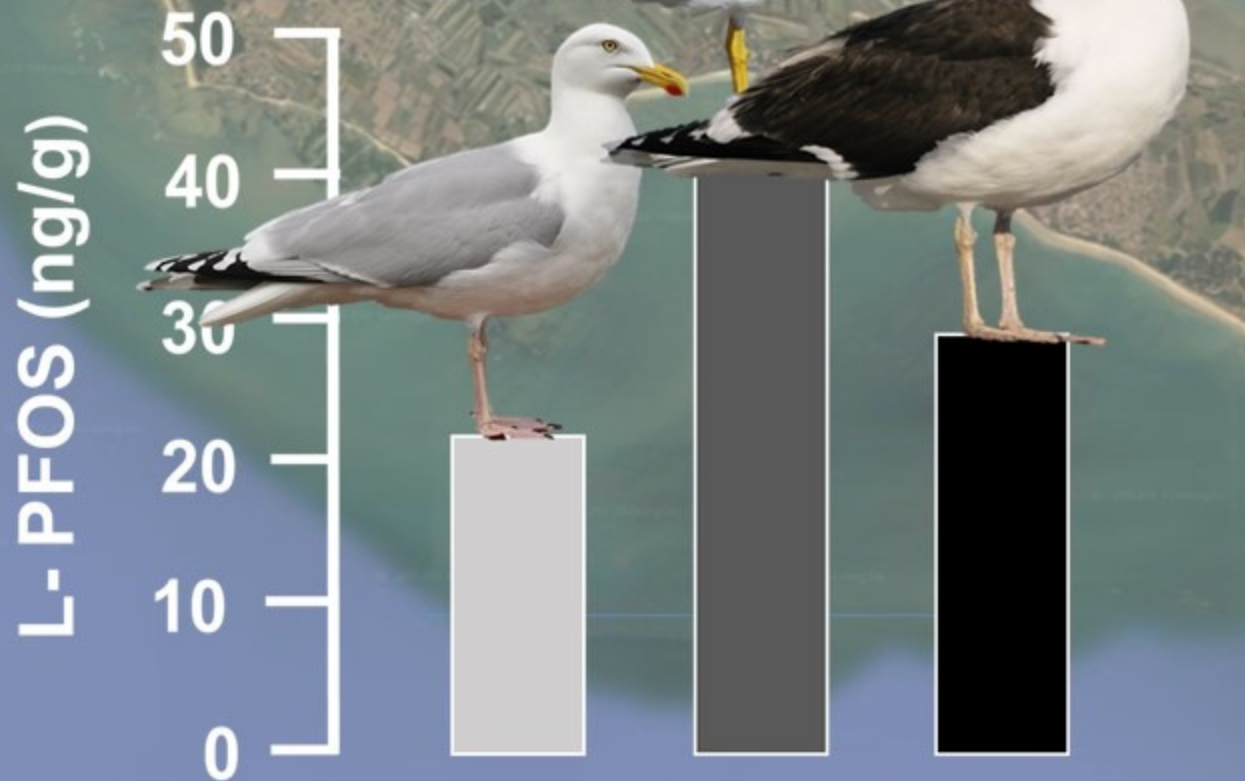
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Ile de Ré (South western France)



Thyroid hormones (T3)

