



## Blood mercury concentrations in four sympatric gull species from South Western France: Insights from stable isotopes and biologging

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1 Blood mercury concentrations in four sympatric gull species from  
2 South Western France: insights from stable isotopes and biologging

3

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25 **ABSTRACT**

26 Mercury (Hg) is a toxic trace element widely distributed in the environment, which  
27 particularly accumulates in top predators, including seabirds. Among seabirds, large gulls  
28 (*Larus sp*) are generalist feeders, foraging in both terrestrial and marine habitats, making  
29 them relevant bioindicators of local coastal Hg contamination. In the present study, we  
30 reported blood Hg concentrations in adults and chicks of four different gull species  
31 breeding on the French Atlantic coast: the European herring gull (*Larus argentatus*), the  
32 Lesser black-backed gull (*L. fuscus*), the Great black-backed gull (*L. marinus*) and the  
33 Yellow-legged gull (*L. michahellis*). We also investigated the potential role of foraging  
34 ecology in shaping Hg contamination across species, using the unique combination of three  
35 dietary tracers (carbon, nitrogen and sulfur stable isotopes) and biologging (GPS tracking).  
36 A high concentration of Hg was associated with high trophic position and a marine diet in  
37 gulls, which was corroborated by birds' space use strategy during foraging trips. Adults of  
38 all four species reached Hg concentrations above reported toxicity thresholds. Specifically,  
39 adults of Great black-backed gulls had a high trophic marine specialized diet and  
40 significantly higher Hg concentrations than the three other species. Blood Hg was 4 to 7  
41 times higher in adults than in chicks, although chicks of all species received mainly marine  
42 and high trophic position prey, which is expected to be the cause of blood Hg  
43 concentrations of toxic concern. By using both stable isotopes and GPS tracking, the  
44 present study provides compelling insights on the main feeding habits driving Hg  
45 contamination in a seabird assemblage feeding in complex coastal environments.

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47 Key words: Contaminant, Trace element, Seabird, Habitat, Movement ecology, Stable  
48 isotopes

## INTRODUCTION

Mercury (Hg) is a non-essential trace element released by both natural and anthropogenic processes (Pirrone et al., 2010). Organic Hg (mostly methylmercury; MeHg) is the main bioavailable and toxic form for living organisms (Díez, 2008; Thompson and Furness, 1989). MeHg is incorporated in organisms mainly via ingestion of contaminated food (Eagles-Smith et al., 2018). In birds, accumulation of MeHg has been associated with a wide range of behavioral and physiological detrimental consequences, impacting individual fitness, and ultimately, population dynamics (Tan et al., 2009; Whitney and Cristol, 2017). Due to a relatively long biological half-life in tissues (Stickel et al., 1977), Hg tends to bioaccumulate in organisms over time and biomagnify up trophic food webs, leading long-lived top predators to exhibit elevated Hg concentrations (Atwell et al., 1998; Cherel et al., 2018; Evers et al., 2005). In coastal environments, seabirds are therefore considered as excellent sentinels of the local contamination (Burger and Gochfeld, 2004; Furness and Camphuysen, 1997; Monteiro and Furness, 1995).

Seabirds show a large interspecific variation in foraging strategies (Ceia and Ramos, 2015), leading to varied degrees of Hg exposure among species (Carravieri et al., 2014b; Monteiro et al., 1998; Stewart et al., 1997). Large gulls of the *Larus* genus are omnivorous and opportunistic feeders. *Larus* spp. are known to exploit both marine and terrestrial habitats, and can scavenge from multiple anthropogenic sources including waste dumps, landfill sites and fishing discards, as well as predating upon eggs or chicks (Buckley, 1990; Mudge and Ferns, 1982; Ramos et al., 2009). Assessing Hg concentrations in sympatric *Larus* species with differences in foraging ecology thus enables to have an overview of the local environmental contamination, particularly in complex coastal

72 habitats (Binkowski et al., 2020). Additionally, in seabirds, individual variation in foraging  
73 behavior appears to be highly prevalent, with individuals differing in their resource use,  
74 habitat selection, and their fidelity to foraging sites (Ceia and Ramos, 2015; Phillips et al.,  
75 2017). Such variation in foraging behavior may be consistent over time, and linked to  
76 individuals' characteristics, such as their age or sex (Bolnick et al., 2003). As Hg exposure  
77 largely varies with feeding strategies and habitat use, this variability shapes intraspecific  
78 differences in Hg contamination (Bustamante et al., 2016; Ceia and Ramos, 2015; Stewart  
79 et al., 1997), with consequences for individual fitness. Characterizing foraging ecology at  
80 the individual level is therefore essential to understand patterns of Hg contamination within  
81 and among species.

82 Tracking devices, including Global Positioning System (GPS) loggers, enable fine  
83 scale monitoring of an animal's movements, which facilitates a direct characterization of  
84 its habitat use (Burger and Shaffer, 2008). Furthermore, recently developed analytical tools  
85 - including hidden Markov models (HMMs) - facilitate behavioral classification of GPS  
86 tracking data (Langrock et al., 2012), enabling the identification of the precise locations  
87 and habitats in which animals are foraging. The analysis of stable isotopes – used as a  
88 proxy for the trophic niche (Newsome et al., 2007) – can also represent an effective tool to  
89 define the foraging habitats (carbon:  $\delta^{13}\text{C}$  and sulfur:  $\delta^{34}\text{S}$ ) and trophic positions (nitrogen:  
90  $\delta^{15}\text{N}$ ) of seabirds (Hobson et al., 1994; Kelly, 2000; Lott et al., 2003). A marine diet is  
91 characterized by higher  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  values, as opposed to lower levels indicating  
92 terrestrial food intakes (Chisholm et al., 1982; Hobson, 1987; MacAvoy et al., 2000;  
93 Schoeninger and DeNiro, 1984). More specifically,  $\delta^{34}\text{S}$  is preferentially used to  
94 differentiate marine vs terrestrial foraging habitats, since it does not present a stepwise

enrichment within the food chain, and therefore shows a greater discriminating power than  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Hobson, 1999; McCutchan Jr et al., 2003). This characteristic makes  $\delta^{34}\text{S}$  particularly interesting to depict seabirds' use of coastal habitats. Therefore, the use of GPS trackers combined with isotopic measurements enables a holistic overview of birds' feeding strategies with respect to multiple available foraging habitats, as well as a high discriminant power (Bracey et al., 2021; Caron-Beaudoin et al., 2013; Ceia et al., 2018; Mendes et al., 2018).

In France, Hg contamination of seabirds has been largely documented in overseas departments and territories (e.g. French Guiana, Sebastiano et al., 2016; 2017; Scattered Islands, Kojadinovic et al. 2007a; Réunion Island, Kojadinovic et al. 2007b; 2007c) and French Southern and Antarctic Territories (e.g., Blévin et al., 2013; Carravieri et al., 2014; 2016; Goutte et al., 2014a; 2014b; Tartu et al., 2014; 2015a). Although the coasts of metropolitan France host large numbers of breeding seabirds (e.g., 174 000 pairs, Cadiou, 2011), data on Hg concentrations in seabird tissues are scarce and limited to chicks of four gull species (*Larus argentatus*, *Larus fuscus*, *Larus marinus* and *Larus michahellis*) from the Southern Bay of Biscay and the English Channel (Binkowski et al., 2020; Zorrozueta et al., 2020). In these studies, high blood Hg concentrations were associated with a diet of marine origin and of high trophic position. Yet, our knowledge on Hg concentrations in other potentially contaminated areas remains very limited.

In the present study, we investigated Hg concentrations in adults and chicks of four gull species from a South Western France colony: Herring gulls *Larus argentatus*, Lesser black-backed gulls *L. fuscus graellsii*, Great black-backed gulls *L. marinus* and Yellow-legged gulls *L. michahellis*. We were particularly interested to i)



describe Hg concentrations of the species during the breeding period; ii) identify the factors driving inter- and intraspecific Hg variations among adults and chicks – in relation to their different feeding habitats inferred from blood  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  stable isotopes, and in relation to their individuals characteristics as age and sex; and iii) explore whether fine-scale habitat partitioning using GPS tracking further elucidates the potential variation in adult Hg contamination. Due to the known differences in foraging ecology among the four species, as well as the individual differences within species, we expected a high inter- and intraspecific variation in Hg concentrations. We further predicted that i) individuals feeding at higher trophic positions (indicated by higher  $\delta^{15}\text{N}$  values) or foraging in marine habitats (indicated by both high  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values, as well as GPS tracking) would exhibit higher Hg concentrations; and that ii) these patterns would persist both between and within species.

## **MATERIALS AND METHODS**

### **Field methodology**

In the breeding seasons of 2016-2019, we sampled gulls breeding in “Lilleau des Niges” National Nature reserve, Ile de Ré, South Western France (46°13'53" N, 1°30'22" W; Figure 1): European herring gulls (EHG, approx. 400 pairs), Lesser black-backed gulls (LBBG, approx. 400 pairs), Great black-backed gulls (GBBG, approx. 200 pairs), and Yellow-legged gull (YLG, approx. 100 pairs). Adult birds were sampled in May ( $n = 140$ ), and chicks in July ( $n = 107$ ). All individuals were captured and sampled only once over the four years. Annual sample sizes for adults and chicks of each species can be found in the Supporting Information (SI) Table S1. Adults incubating a full-size clutch (i.e. 2 to 3 eggs)

were caught on their nests, using a trap placed over the eggs. Chicks of approximately 1 month of age were caught by hand, within the colony. From an early age, gull chicks are developed enough to explore their environment and do not stay in their nest, therefore we were unable to assign relatedness among chicks and adults. Skull and tarsus were measured using a caliper ( $\pm 0.1$  mm). Wing length was also measured using a ruler ( $\pm 1$  mm), and birds were weighted using a Pesola spring balance ( $\pm 5$ g). Blood samples (2 mL,  $<1$  % of the body mass of LBBG, the smallest species in this study) were taken from the brachial vein of adults and chicks using heparinized syringes. Blood was transferred into a 2 mL Eppendorf<sup>®</sup> tube and immediately stored in a cool box. At the end of each sampling day, whole blood was centrifuged and red blood cells (hereafter “blood”) were stored at  $-20^{\circ}\text{C}$  until laboratory analyses. Blood samples were then used to measure Hg and stable isotopes and for molecular sexing.

#### **Mercury analysis**

The analysis of total Hg in lyophilized blood was carried out using an Altec AMA 254 spectrophotometer following Bustamante et al. (2006). Each sample (mass:  $\sim 0.5$  mg dry weight, dw) was analysed twice and Hg concentrations were then averaged. For each individual, the relative standard deviation was  $<10$  %. Accuracy was checked at the beginning and the end of each measurement session using a certified reference material (2016-2018: DOLT-5 dogfish liver - Hg certified concentration (mean  $\pm$  SD):  $0.44 \pm 0.18$   $\mu\text{g g}^{-1}$  dw; 2019: TORT-3 lobster hepatopancreas - Hg certified concentration:  $0.29 \pm 0.02$   $\mu\text{g g}^{-1}$  dw; obtained from NRC, Canada). Recoveries were respectively  $0.422 \pm 0.005$  and  $0.318 \pm 0.014$   $\mu\text{g g}^{-1}$  dw. Blanks were also run before and after each set of samples. The

limit of detection of the AMA was 0.1 ng. Hg concentrations are expressed as  $\mu\text{g g}^{-1}$  dw. In several bird species, MeHg represents most of the total Hg in blood (Renedo et al., 2018; Rimmer et al., 2005). Chicks accumulate Hg from hatching, being fed by their parents from local food items. For this reason, they are relevant bioindicators of the local environment contamination (Binkowski et al., 2020; Blévin et al., 2013). By contrast to chicks, using adult gulls as bioindicators of their local environment during breeding is still debated as the half-life of Hg in avian tissues is 1~3 months, and contaminants acquired during migration might still be present in blood (Bearhop et al., 2000; Monteiro and Furness, 2001; Stickel et al., 1977). However, all species used in the present study were on the breeding grounds more than 3 months before sampling, therefore blood Hg should mainly represent local contamination.

#### **Stable isotope analysis**

Sub-samples of blood were weighed (mean  $\pm$  SD:  $0.29 \pm 0.06$  mg dw for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ;  $0.71 \pm 0.05$  mg dw for  $\delta^{34}\text{S}$ ) with a microbalance and then packed into tin containers for combustion. Relative abundances of carbon, nitrogen and sulfur isotopes were measured using a continuous flow mass spectrometer (Thermo Scientific Delta V Advantage) coupled with an elemental analyzer (carbon and nitrogen: Thermo Scientific Flash EA 1112; sulfur: Thermo Scientific Flash IRMS EA IsoLink). The delta ( $\delta$ ) notation relative to Vienna PeeDee Belemnite, atmospheric  $\text{N}_2$  and Vienna Cañon Diablo troilite for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  respectively, was used to present isotopic results. Accuracy was checked by replicate measurements of the internal laboratory standard, and analytical precision was  $<0.10$  ‰ for  $\delta^{13}\text{C}$ ,  $<0.15$  ‰ for  $\delta^{15}\text{N}$  and  $<0.20$  ‰ for  $\delta^{34}\text{S}$  values. The stable

isotopes values were computed as  $\delta X = \left[ \left( \frac{R_{Sample}}{R_{Standard}} \right) - 1 \right] \times 1000$ , where X stands for  $^{13}\text{C}$ ,  $^{15}\text{N}$  or  $^{34}\text{S}$  and  $R$  being the ratio  $^{13}\text{C}/^{12}\text{C}$ ,  $^{15}\text{N}/^{14}\text{N}$  or  $^{34}\text{S}/^{32}\text{S}$ . Turnover time for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in blood is 11~30 days (Barquete et al., 2013; Hobson and Clark, 1992; 1993), therefore stable isotopes values are representative of the breeding period in adults.

### **GPS tracking analysis**

A subset ( $n = 43$ ) of the captured adults (18 EHG, 18 LBBG and 7 GBBG, see SI Table S1) were equipped with GPS-UHF loggers (HARRIER, Ecotone®) in 2017 and 2018. Among the 43 GPS equipped birds, 17 EHG, 18 LBBG and 6 GBBG were blood sampled and included in Hg and stable isotopes analyses. The total mass of the logger and the harness (used for attachment) weighted on average 13g (i.e. <2 % of the body mass of LBBG, the smallest species in this study).

GPS loggers were set to record locations every 5 min. For each tracked individual, locations were filtered to the period between the day after blood sampling to the last day before either nest failure or egg hatching had been recorded (Figure 1 & SI Figure S1 & Table S2). Foraging sites were identified from GPS tracks by assigning a behavioral state to each location using Hidden Markov Models (hereafter “HMMs”, Franke et al. 2004) fitted using the moveHMM R package (Michelot et al., 2016). Additional information on the spatial data treatment and the behavioral classification can be found in the SI. The habitat for each of these locations was assigned as either “pelagic”; “coastal” (i.e. lagoons, estuaries, coastal salt marshes, intertidal flats), or “terrestrial” (all others) based on the CORINE Land Cover dataset (CORINE Land Cover; Feranec et al. 2016). Ratios of pelagic vs coastal and marine (i.e., pelagic and coastal) vs terrestrial habitats used by each

individual were then calculated.

For an overview of the feeding behaviors leading to the observed Hg concentrations in the tracked species, we estimated birds' foraging site fidelity. As well as giving important insight into the degree of specialization in birds' uses of foraging habitat, this measure of site fidelity served to test whether individuals' foraging behavior in the period after blood sampling was likely to be representative of their behavior prior to blood sampling (see SI). Individual foraging site fidelity was assessed following the randomization procedure of Harris et al. (2020). For each foraging site, the distance to a randomly selected site from the same individual during another trip (within-individual comparison), as well as to a randomly selected site from each other individual of the same species (between-individual comparisons) was measured. The site fidelity index corresponds to the proportion of between-individual sites that were farther from the focal site than the within-individual site. This index is bounded between 0 and 1, with high values representing high site fidelity (an individual forages more closely to its own foraging sites than to the sites of other individuals), and low values representing low site fidelity (an individual forages more closely to the foraging sites of other individuals than to its own foraging sites). Additional information can be found in the SI.

### **Molecular sexing**

All adults and chicks ( $n = 247$ ) were sexed using blood samples by polymerase chain reaction (PCR) amplification of parts of two highly conserved genes (CHD) of sex chromosomes, following Fridolfsson and Ellegren (1999).

## Statistical analyses

Statistical analyses were performed using R 4.0.0 (R Core Team, 2020). One YLG chick was removed from the dataset due to outliers in  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values. In all species, adults had much higher Hg concentrations than chicks, and therefore chicks and adults were treated separately.

Firstly, relationships between Hg concentrations and diet (inferred by stable isotope values) were investigated. As  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  were positively correlated in adults (Pearson correlation  $>0.70$ ,  $p < 0.001$  for all relationships) and partially correlated in chicks (correlation: C-N = 0.72,  $p < 0.001$ ; S-C = -0.15; S-N = -0.18, both  $p > 0.05$ ), principal components analyses (“FactoMineR” R package, version 2.3; Lê et al., 2008) were performed on the three isotopes to reduce the number of explanatory variables. Principal components (PCs) which explained more than 80 % of the total variance were retained. In adults, principal components analysis reduced  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  to one PC (PC<sub>1</sub>) explaining 83.6 % of the variance and equally influenced by  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  (SI Figure S2 & Table S3). High PC<sub>1</sub> scores reflected high  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$ . In chicks, principal components analyses reduced  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  to two PCs explaining 92.0 % of the variance (SI Figure S2 & Table S3). High PC<sub>1</sub> scores indicated high  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, and high PC<sub>2</sub> scores indicated high  $\delta^{34}\text{S}$  values. The isotopic niches, which are essential for an overview of each species’ local feeding habits, were investigated, and the foraging ecology of each species were illustrated and described in the SI. Generalized least square (GLS) models were then used to investigate the effects of the selected PCs on Hg (“nlme” R package, version 3.1-151; Pinheiro et al., 2020). For that, using Hg as the response variable, we included as explanatory covariates the selected PCs and the species. Year was

not included as a predictor in the models to improve statistical power, and also because four years of sampling was insufficient to evaluate temporal trends in Hg concentration. We used GLS models instead of linear models (LM) due to heteroscedasticity in Hg concentrations among species (see SI for further information). For both adults and chicks, after running GLS models, Tukey post-hoc pairwise comparison tests were performed to quantify the differences in Hg concentrations among species (“emmeans” R package, version 1.7.1-1; Lenth et al., 2021). Although performed on only three variables, PCA enabled the inclusion of ecological information provided by the three stable isotopes rather than exploring the effect of each stable isotopes in independent models (similar conclusions were obtained using both methods, see SI)

Secondly, as Hg levels were found to be highly variable among species, we ran one LM per species to investigate differences between the sexes. With Hg as the response variable, we included the sex as an explanatory variable. This model was not fitted for adults of YLG as only a single female was sampled. In previous studies, chick age has been found to be a key predictors of Hg contamination, as blood Hg strongly fluctuates during chick growth, reaching highest concentrations when feathers stop growing (Spalding et al., 2000). Morphology is commonly used as a proxy of chick age, and we therefore also included the skull length (head and bill) in the LMs for chicks as an additional explanatory variable. Where Hg differed significantly between sexes, an additional LM was built to investigate whether this was linked to sex differences in diet, with the selected PCs fitted as the response and sex as an explanatory variable.

Finally, in GPS-tracked adults, the impact of the use of marine or terrestrial habitats on Hg concentrations was investigated using a GLS model: Hg was fitted as the response

variable, and the proportional use of marine vs terrestrial habitat and species (to control for the variation among species) were included as explanatory covariates. A similar model was built to investigate the impact of the use of coastal or pelagic habitats on Hg concentrations, including coastal vs pelagic habitats and species (to control for the variation among species) as explanatory covariates. Before modelling, two individuals that were tracked but not blood sampled were removed from the dataset.

For all models, a set of candidate models including all possible combinations of the predictors, ranging from the full model (that includes each of the stated variables) to the null model was built. In each case, the best candidate model – i.e., the one with the lowest second-order Akaike’s Information Criterion value for small sample size (AICc; “AICcmodavg” R package, version 2.2-2; Mazerolle, 2017), or the most parsimonious model among those with a  $\Delta AICc \leq 2$  – was retained for inference. Homogeneity and normality of the residuals were visually checked using plots of residuals vs fitted values and histograms of the residuals (Zuur et al., 2007). In post-hoc tests, an  $\alpha < 0.05$  threshold was used to assess the significance of the tests.

## RESULTS

### Mercury in relation to feeding habits as inferred by stable isotopes

In chicks, Hg concentrations ranged from  $0.27 \mu\text{g g}^{-1} \text{ dw}$  in EHG to  $3.86 \mu\text{g g}^{-1} \text{ dw}$  in GBBG (Table 1). Model selection indicated that  $PC_1$  and species were significant predictors of Hg concentrations, but not  $PC_2$  (SI Table S4). Post-hoc comparison test showed that GBBG had the highest Hg concentrations, followed by YLG that was not significantly different from EHG and LBBG, but LBBG had lower Hg concentrations than



EHG (Tables 1 & 2, Figure 2). Chicks also exhibited a positive relationship between Hg concentration and PC<sub>1</sub> (estimate  $\pm$  SE =  $0.09 \pm 0.03$ ,  $t = 3.49$ ,  $p < 0.001$ ; Table 2 & Figure 3). Sex was not retained as a significant predictor of Hg concentration for any species, but skull size (as a proxy for chick age) was included in the best model for GBBG only (SI Table S5), with Hg significantly increasing with skull size in this species ( $F_{1, 26} = 6.51$ ,  $p = 0.017$ ; estimate  $\pm$  SE =  $0.04 \pm 0.02$ ).

In adults, individual blood Hg concentrations ranged from  $0.89 \mu\text{g g}^{-1}$  dw in EHG to  $21.5 \mu\text{g g}^{-1}$  dw in GBBG (Table 1). Model selection showed that PC<sub>1</sub> and species were significant predictors of Hg concentrations in adult gulls (SI Table S4). Post-hoc comparison test indicated that GBBG had the highest Hg concentrations, while lower concentrations were found in YLG, LBBG and EHG (not significantly different among them; Tables 1 & 2, Figure 2). Adults also showed a significant and positive association between Hg concentration and PC<sub>1</sub> (estimate  $\pm$  SE =  $0.55 \pm 0.06$ ,  $t = 9.25$ ,  $p < 0.001$ ; Table 2 & Figure 3). Sex was retained for GBBG only (SI Table S5), with males having higher Hg concentrations than females in adults of this species ( $F_{1, 35} = 19.2$ ,  $p < 0.001$ ; estimate  $\pm$  SE =  $4.62 \pm 1.05$ ). However, sex was not retained as a significant predictor of stable isotopes in GBBG (PC<sub>1</sub>; SI Table S6).

### **Mercury in relation to habitat use as inferred by GPS tracking**

From GPS tracked adults, a total of 1069 foraging trips from 43 individuals were recorded (612 in EHG, 304 in LBBG and 154 in GBBG). The three tracked species showed different patterns of habitat use: GBBG forage predominantly at sea and showed low inter-individual variation (average use of marine habitats  $\pm$  SD:  $0.93 \pm 0.05$  %), while EHG ( $0.63$

325  $\pm 0.23$  %) and LBBG ( $0.78 \pm 0.17$  %) individuals were more spread on a gradient from  
326 strictly terrestrial to strictly marine (Figure 1 & SI Figure S3). The use of marine habitats  
327 in EHG and GBBG was mainly coastal (proportion of coastal habitats use  $\pm$  SD:  $0.58 \pm$   
328  $0.25$  and  $0.90 \pm 0.07$  respectively), while LBBG used pelagic and coastal habitats  
329 (proportion of coastal habitats use  $\pm$  SD:  $0.23 \pm 0.16$ ; Figure 1 & SI Figure S3). Model  
330 selection indicated that the proportional use of marine vs terrestrial habitats and the species  
331 were significant predictors of Hg variations (SI Table S7), Hg concentrations increased  
332 with the use of marine habitats in all three species (Estimate  $\pm$  SE =  $2.59 \pm 0.99$ ,  $t = 2.62$ ,  
333  $p = 0.01$ ; Figure 4). Conversely, the coastal vs pelagic gradient was not a significant  
334 predictor of Hg variations in the three gull species. (Table S7). GBBG, EHG and LBBG  
335 exhibited a relatively high fidelity to foraging sites (fidelity index  $\pm$  SD =  $0.71 \pm 0.18$ ;  $0.59$   
336  $\pm 0.13$  and  $0.72 \pm 0.13$  respectively).

337

## 338 **DISCUSSION**

339 Blood Hg concentrations of four sympatric gull species were investigated in  
340 relation to their foraging ecology inferred from stable isotopes  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  blood  
341 values in adults and chicks, and from GPS tracking of adult birds. High Hg concentrations  
342 were related to stable isotopes values representative of a high trophic position food, mainly  
343 of marine origin. Foraging movements tracked during breeding corroborated the  
344 relationship between Hg and the birds' foraging strategies with relation to terrestrial vs  
345 marine habitat use. All species showed foraging site fidelity, indicating a degree of  
346 specialization in their use of foraging sites, and thus high potential for consistent individual  
347 differences in contaminant level to emerge. This led some adult individuals to exceed

reported toxicity thresholds in all four species, particularly in GBBG, showing extremely high levels of Hg. These findings are also concerning for chicks, which predominantly receive marine food from their parents, and are therefore likely to accumulate high concentrations of Hg. Together, these results support the use of sympatric species of gulls as bioindicators of contamination in complex coastal habitats.

### **Mercury concentrations**

LBBG and GBBG chicks had blood Hg concentrations similar to those from the French coast of the English Channel, although EHG chicks had higher Hg concentrations, even compared to the colonies from the Seine estuary, one of the most polluted rivers in Europe (Binkowski et al., 2020; Cossa et al., 2002). Toxicological benchmarks are usually assessed for adult birds, meaning that chicks Hg concentrations can only be compared to published thresholds from adults with caution. However, much lower blood Hg concentrations have been associated with an impaired physiological condition in LBBG chicks (lower metabolism markers, increased oxidative stress and increased anaerobic metabolism; Santos et al., 2020). In the present study, all four species showed similar Hg levels between sexes, likely because sex difference in contaminant exposure is usually visible later in life. However, in GBBG we detected increased levels of Hg with skull size (a proxy of chick age), but this result was not found in other species. Blood Hg is known to increase with the chicks age when most of the feathers have grown (Ackerman et al., 2011). This relationship may be easier to detect in GBBG as they grow larger, thus their among individual variation in size is higher than the other investigated species.

Hg concentrations in adults ranged from similar (EHG and LBBG) to much higher (YLG and GBBG) than in gulls from the Baltic Sea including EHG and GBBG, or Brown skuas (*Stercorarius antarcticus lonnbergi*) from three colonies of the Southern Ocean (Mills et al., 2022; Szumiło-Pilarska et al., 2017). Notably, we found GBBG to have average blood Hg concentrations similar to those of the Wandering albatross (*Diomedea exulans*; Anderson et al., 2009), the highest trophic position seabird in the Southern Ocean (Cherel et al., 2017) and one of the species with the highest known Hg levels worldwide (Blévin et al., 2013; Carravieri et al., 2014a; 2014b; Cherel et al., 2018). Such Hg concentrations are known to significantly impact reproduction, including breeding success in various bird species (Goutte et al., 2015; Tartu et al., 2013; 2014; 2015b; Ackerman et al., 2016). As no physiological endpoints were measured in the present study, it is not possible to directly draw conclusions on the impact of Hg concentrations of this population. Other factors may also contribute to a reduced toxicity of Hg. For instance, selenium – which was not measured in the present study – is an essential trace element which may protect birds against Hg toxicity (Dietz et al., 2000; Manceau et al., 2021). However, Hg is known to affect almost all aspects of avian physiology and life history traits (Sebastiano et al., 2022; Tartu et al., 2015a; Whitney and Cristol, 2017). We therefore emphasize further work to investigate whether the reported high blood Hg concentrations are detrimental to the different species of birds in the region. Similarly to what we observed in GBBG, higher concentrations of Hg in males than in females were previously found in other bird species (Ackerman et al., 2007; Burgess et al., 2005; La Sala et al., 2011). Interestingly, incubating GBBGs males and females were not found to differ in terms of stable isotopes concentration, suggesting diet is not the main driver of this difference. A possible

explanation may be represented by the fact that female gulls deposit Hg into their eggs therefore lowering their circulating contaminants (Lewis et al., 1993).

### **Mercury concentrations in relation to foraging ecology**

The positive relationship between blood Hg concentrations and  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values observed in gull chicks is in agreement with previous studies on the same four species in the English Channel (Binkowski et al., 2020) and the southern Bay of Biscay (Zorrozueta et al., 2020). However, blood  $\delta^{34}\text{S}$  was not found to be an important driver of Hg contamination at Ile de Ré, contrary to chicks in the English Channel (Binkowski et al., 2020) and Spain (Ramos et al., 2013). In the present study, chick isotopic values were in the highest range values of those of their parents for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$ , meaning that they predominantly received food at a higher trophic position and of marine origin. A diet shift after hatching – reflecting different food requirement before and after hatching – has previously been described in EHG and other species of gulls (Annett and Pierotti, 1989; Bukacińska et al., 1996; Pierotti and Annett, 1987). This specialized chick diet of low marine prey diversity, provided by the parents, may explain why the terrestrial vs marine gradient is not a good predictor of Hg variations in chicks.

Isotopic data showed that, similarly to chicks, blood Hg contamination was higher in adult gulls feeding on marine than terrestrial items. This is consistent with previous studies investigating the relationship between Hg and  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  in coastal seabirds and their prey (Gongora et al., 2018; Peterson et al., 2017). These patterns likely reflect the biomagnifying characteristics of Hg along food webs, with longer and more complex food webs in the marine environment (Bełdowska and Falkowska, 2016; Morel et al., 1998), as

416 well as that MeHg is mainly produced in the ocean (Chen et al., 2008). Conversely, some  
417 previous studies did not report such relationship in other aquatic birds (Einoder et al., 2018;  
418 Soldatini et al., 2020), however, the association between Hg and  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  is complex  
419 and largely depends on the species or their environments. Different environments likely  
420 differ in their  $\delta^{15}\text{N}$  baseline (Elliott et al., 2021), thus precluding comparing directly  
421 consumers'  $\delta^{15}\text{N}$  values as reflecting their trophic position. This is likely the case of gulls  
422 from Ile de Ré, meaning that any blood  $\delta^{15}\text{N}$  difference at the species or individual level  
423 must be interpreted with caution, especially if their  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values indicate foraging  
424 in different habitats.

425 In the present study, EHG, LBBG and YLG showed important inter-individual  
426 variations in blood  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  values, that characterize populations of generalist  
427 species made of specialist individuals (Jaeger et al., 2009). In contrast, GBBG exhibited  
428 narrower ranges for all isotopic values, representative of a specialist species. GBBG's  
429 isotopic values were in the highest range of those of the other gull species, that characterize  
430 a diet based on marine items ( $\delta^{34}\text{S}$ : +15 to +19 ‰), whereas EHG, LBBG and YLG  
431 individuals showed a diet of mixed terrestrial, intertidal and marine sources (Nehlich, 2015;  
432 Rees et al., 1978). The very high Hg concentrations found in GBBG are therefore most  
433 likely related to its marine diet. These isotopic results are supported by the analyses of  
434 foraging trips in EHG, LBBG and GBBG, as the use of a marine habitats was related to  
435 higher blood Hg concentrations. In other species of coastal gulls equipped with tracking  
436 devices, including the American Herring gull (*Larus smithsonianus*) and the Western gull  
437 (*Larus occidentalis*), a similar pattern was recently observed, with individuals mainly  
438 foraging in marine environments exhibiting the highest Hg concentrations (Clatterbuck et

al., 2021; Thorne et al., 2021). EHG showed higher use of coastal foraging sites but similar Hg concentrations compared to the more pelagic LBBG. The coastal specialists GBBG also presented the highest Hg concentrations, further suggesting that “marine vs terrestrial” may represent a better gradient than “coastal vs pelagic” to explain Hg variations in gull species. As  $\delta^{34}\text{S}$  highly discriminates between terrestrial and marine habitats (McCutchan Jr et al., 2003), we further stress the importance of the use of  $\delta^{34}\text{S}$  as relevant index of foraging habitats.

Nevertheless, Figures 3 & 4 reveal a weaker relationship between blood Hg and  $\text{PC}_1$  in GBBGs. It was however not possible to include the interaction between  $\text{PC}_1$  and species in the models to study if this relationship was different among species, due to the relatively different range in  $\text{PC}_1$  and Hg values between GBBG and the other species. In chicks, the additive effect of maternal Hg initially transferred in the egg may contribute to blur the relationship in all species.

Surprisingly, the range of Hg concentrations seems to be inversely correlated to the isotopic niche width in adults, i.e., with the foraging habitat diversity. In the specialist GBBG, high fidelity to foraging sites may explain the high inter-individual variation in Hg concentration despite a narrow isotopic niche, i.e., birds feeding on a similar trophic position prey but specialized in areas of different contamination intensities (river estuarine, salt-marshes, intertidal area, open sea,...; Mitchell and Gilmour, 2008), generating individual variation in Hg exposure. EHG and LBBG also exhibited a relatively high fidelity to foraging sites, but the lower range of Hg concentrations compared to GBBG suggests that the foraging areas provided by movement analyses are important to understand the sources of Hg in specialist species.

462

## 463 **CONCLUSION**

464         In the present study, we have highlighted the need to refine ecotoxicological studies  
465 with stable isotope analyses, as the trophic position (using  $\delta^{15}\text{N}$  as a proxy) and the feeding  
466 habitat ( $\delta^{34}\text{S}$ ) were important drivers of blood Hg contamination. We also point out that not  
467 only the use of GPS data highly reflects the results on Hg concentrations and habitat  
468 selection through isotopic analyses, but it may further provides fundamental insights on  
469 inter-individual contamination through habitat fidelity. We argue for the concomitant use  
470 of both GPS and the stable isotopes method to characterize the sources of contaminants in  
471 seabirds.

472         Our results also call for a potential toxicological risk in both chicks and adults. As  
473 chicks receive high trophic position prey of marine origin from their parents, such diet  
474 could lead to Hg concentrations of concern during their development. In adults, GBBG  
475 showed particularly high concentrations of Hg due to its almost exclusively marine food.  
476 Nonetheless, several adults of all four species might be at risk as exceeded previously  
477 reported Hg toxicity thresholds. It is therefore fundamental to further investigate the  
478 potential detrimental effects of Hg exposure on physiological and life-history traits of the  
479 studied species.

480

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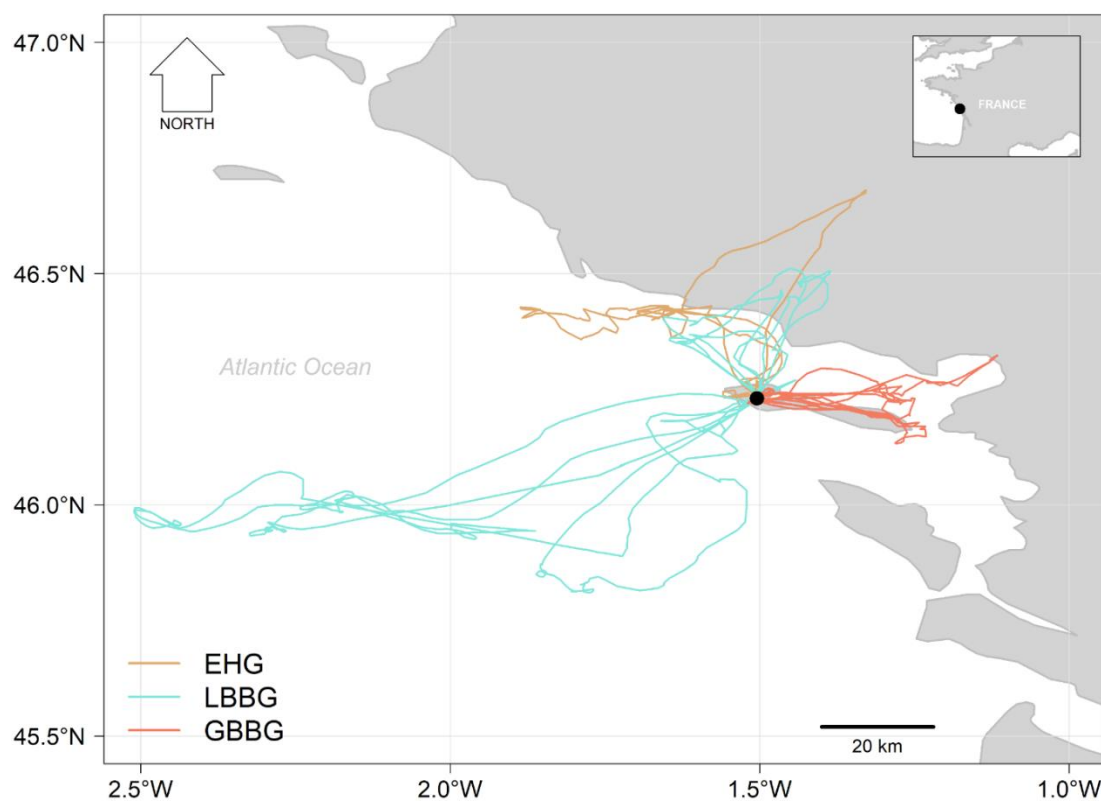
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502 **Figure 1.** Map of the Ile de Ré and surroundings, France, visited by the adults of four species of gulls from the “Lilleau des Niges”  
503 colony during their breeding period; European herring gulls (EHG), Lesser black-backed gulls (LBBG), Great black-backed gulls  
504 (GBBG) and Yellow-legged gulls (YLG). The studied colony is represented by the black point. YLG were not tracked and so are not  
505 presented, but were included in analyses of Hg and stable isotopes. Tracks presented here are a random selection of 5 trips per species,  
506 see SI Figure S1 for maps of all tracks per species.



**Table 1.** Hg concentrations ( $\mu\text{g g}^{-1}$  dw) and carbon ( $\delta^{13}\text{C}$ ), nitrogen ( $\delta^{15}\text{N}$ ) and sulfur ( $\delta^{34}\text{S}$ ) stable isotope values (‰) measured in blood of adults and chicks of four gull species from the Ile de Ré, France: sample size ( $n$ ), mean  $\pm$  standard deviation ( $SD$ ), median and range (min-max). For adults, Hg values are presented separately for females ( $\text{♀}$ ) and males ( $\text{♂}$ ).

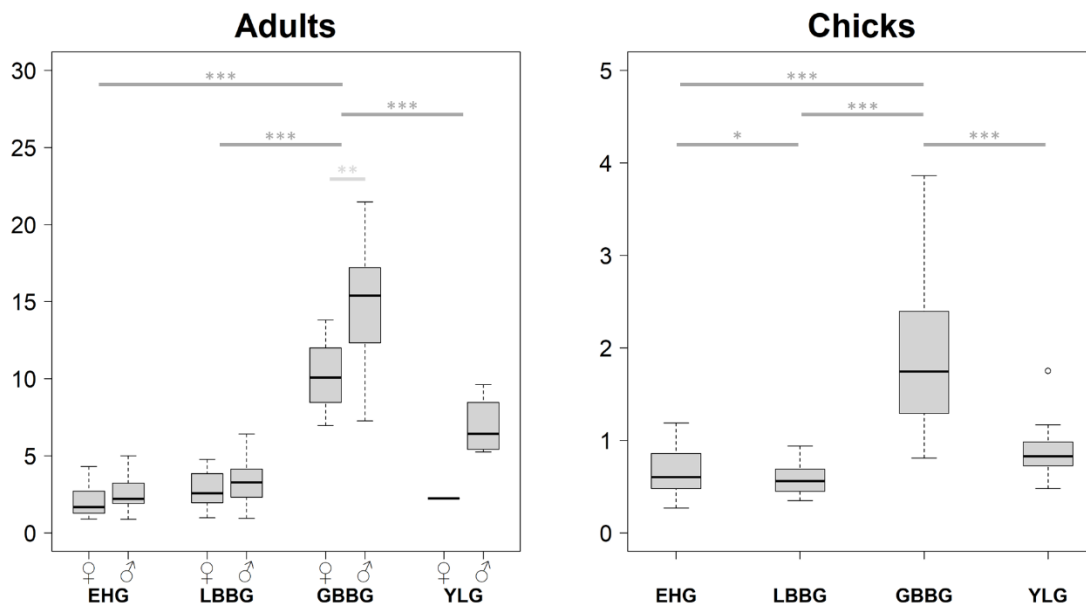
Adults									
	$n$	Hg		$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		$\delta^{34}\text{S}$	
		Mean $\pm$ SD	Min/max	Mean $\pm$ SD	Min/max	Mean $\pm$ SD	Min/max	Mean $\pm$ SD	Min/max
EHG ( <i>Larus argentatus</i> )	44	2.29 $\pm$ 1.06	0.89/5.01	-19.4 $\pm$ 1.93	-22.9/-15.5	12.7 $\pm$ 1.82	9.76/16.0	13.1 $\pm$ 2.23	8.72/17.0
♀	23	2.05 $\pm$ 0.96	0.90/4.31						
♂	21	2.55 $\pm$ 1.12	0.89/5.01			-			
LBBG ( <i>Larus fuscus</i> )	54	3.08 $\pm$ 1.31	0.95/6.42	-19.1 $\pm$ 1.34	-22.6/-17.6	13.8 $\pm$ 1.30	10.9/15.8	15.9 $\pm$ 2.37	9.90/18.6
♀	27	2.82 $\pm$ 1.03	0.98/4.78						
♂	27	3.35 $\pm$ 1.52	0.95/6.42			-			
GBBG ( <i>Larus marinus</i> )	37	12.5 $\pm$ 3.93	6.97/21.5	-16.8 $\pm$ 0.83	-19.7/-14.7	16.4 $\pm$ 0.41	15.8/17.8	16.9 $\pm$ 1.07	14.6/18.7
♀	19	10.3 $\pm$ 2.20	6.97/13.8						
♂	18	14.9 $\pm$ 4.00	7.26/21.5			-			
YLG ( <i>Larus michahellis</i> )	5	6.00 $\pm$ 2.72	2.25/9.63	-19.4 $\pm$ 3.51	-25.2/-16.7	15.1 $\pm$ 1.32	12.76/15.91	12.6 $\pm$ 4.66	5.87/16.7
♀	1	2.25 $\pm$ NA	2.25/2.25						
♂	4	6.94 $\pm$ 2.00	5.26/9.63			-			
Chicks									
	$n$	Hg		$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		$\delta^{34}\text{S}$	
		Mean $\pm$ SD	Min/max	Mean $\pm$ SD	Min/max	Mean $\pm$ SD	Min/max	Mean $\pm$ SD	Min/max
EHG ( <i>Larus argentatus</i> )	30	0.68 $\pm$ 0.25	0.27/1.19	-18.3 $\pm$ 0.66	-19.9/-17.1	13.8 $\pm$ 0.75	12.1/15.0	16.8 $\pm$ 1.21	14.0/18.7
LBBG ( <i>Larus fuscus</i> )	37	0.58 $\pm$ 0.15	0.35/0.94	-18.3 $\pm$ 0.27	-18.8/-17.7	14.5 $\pm$ 0.41	13.7/15.2	18.4 $\pm$ 0.93	16.7/19.8
GBBG ( <i>Larus marinus</i> )	28	1.91 $\pm$ 0.76	0.81/3.86	-17.1 $\pm$ 0.77	-19.33/-16.7	15.6 $\pm$ 0.48	14.0/16.6	16.5 $\pm$ 1.32	12.8/18.6
YLG ( <i>Larus michahellis</i> )	11	0.90 $\pm$ 0.34	0.48/1.75	-18.0 $\pm$ 0.61	-19.37/-17.42	14.9 $\pm$ 1.07	12.4/16.1	16.5 $\pm$ 1.46	14.07/18.4

**Table 2.** Outputs of linear models examining the variables affecting Hg concentration in four gull species from the Ile de Ré, France: European herring gulls (EHG), Lesser black-backed gulls (LBBG), Great black-backed gulls (GBBG) and Yellow-legged gulls (YLG). Significant *p*-values are in bold.

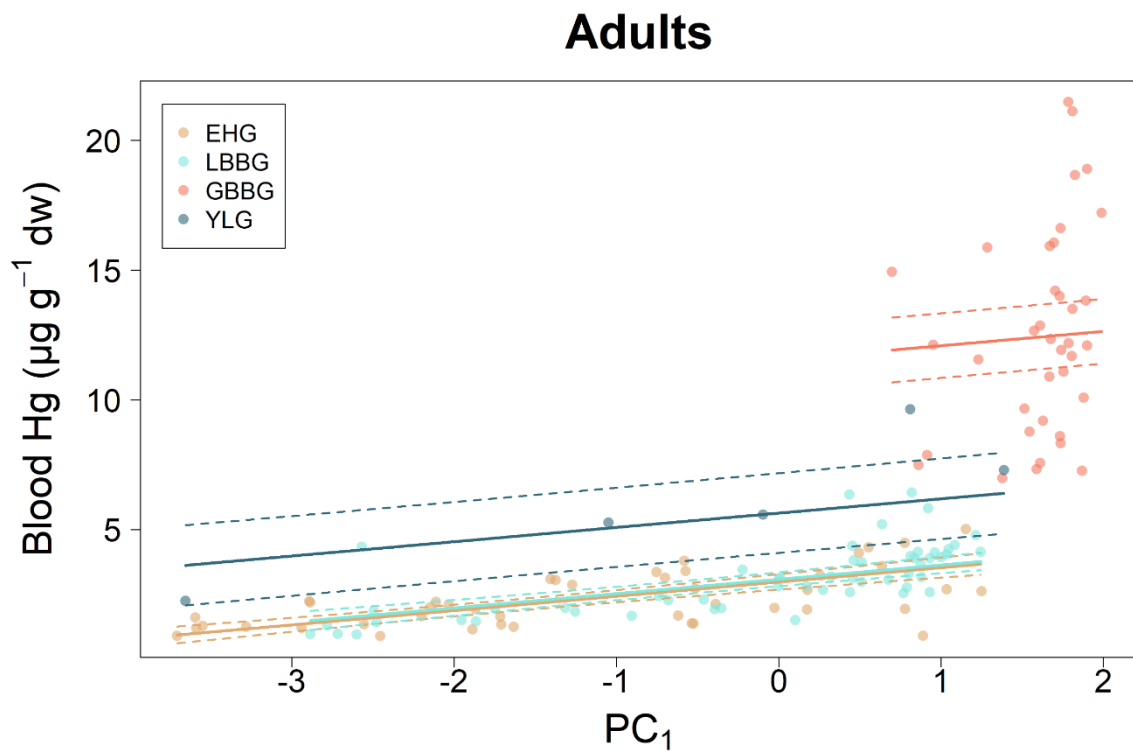
Adults				
Parameter	Estimate	SE	<i>t</i> -value	<i>p</i> -value
PC <sub>1</sub>	0.55	0.06	9.25	<b>&lt;0.001</b>
EHG - LBBG	0.10	0.19	0.56	0.943
EHG - GBBG	8.56	0.67	12.70	<b>&lt;0.001</b>
EHG - YLG	2.65	0.79	3.38	0.089
LBBG - GBBG	8.45	0.66	12.79	<b>&lt;0.001</b>
LBBG - YLG	2.55	0.79	3.22	0.099
GBBG - YLG	-5.90	1.02	-5.78	<b>&lt;0.001</b>

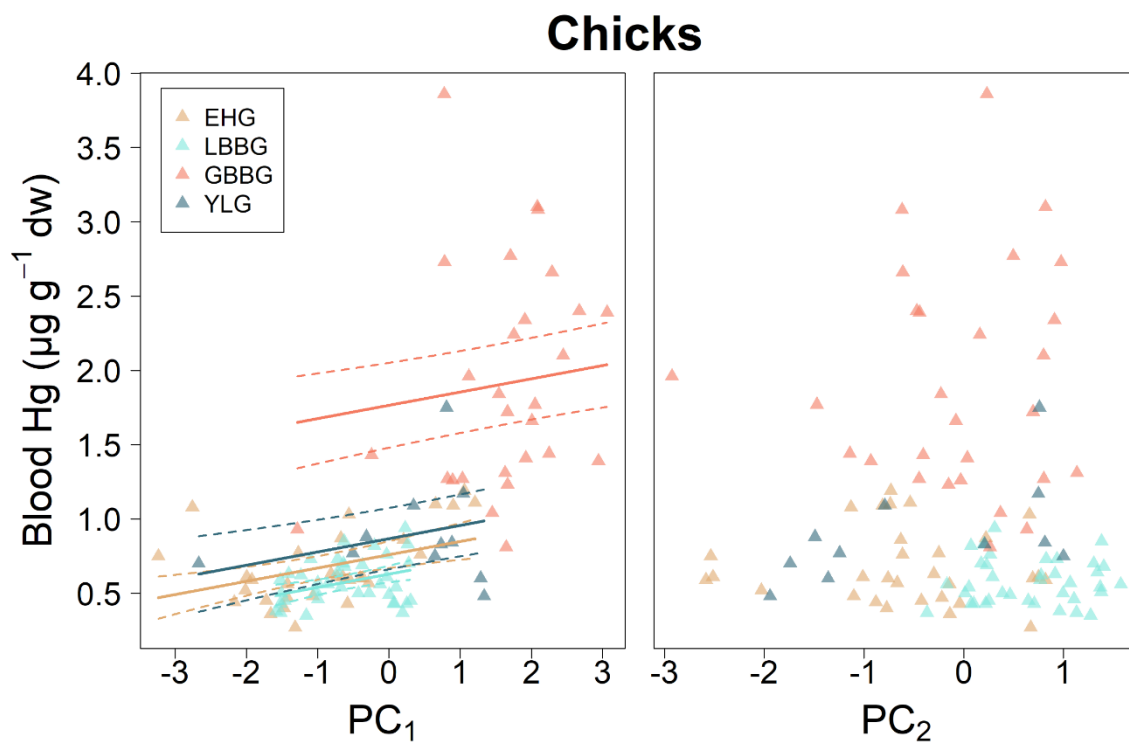
Chicks				
Parameter	Estimate	SE	<i>t</i> -value	<i>p</i> -value
PC <sub>1</sub>	0.09	0.03	3.49	<b>&lt;0.001</b>
EHG - LBBG	-0.13	0.05	-2.72	<b>0.043</b>
EHG - GBBG	1.01	0.16	6.33	<b>&lt;0.001</b>
EHG - YLG	0.11	0.12	0.93	0.792
LBBG - GBBG	1.14	0.15	7.45	<b>&lt;0.001</b>
LBBG - YLG	0.24	0.11	2.16	0.189
GBBG - YLG	-0.90	0.18	-5.05	<b>&lt;0.001</b>

518 **Figure 2.** Blood concentrations of Hg in adults and chicks of four gull species from the  
 519 Ile de Ré, France; European herring gulls (EHG), Lesser black-backed gulls (LBBG),  
 520 Great black-backed gulls (GBBG) and Yellow-legged gulls (YLG). For adults (left), Hg  
 521 values are shown separately for females (♀) and males (♂). Interspecific (dark-grey) and  
 522 between sex (light grey) statistically significant differences are indicated by the asterisk:  
 523 \*, \*\*, \*\*\*; indicating a *p*-value < 0.05, <0.01, and <0.001, respectively. Values are  
 524 median, 25<sup>th</sup> and 75<sup>th</sup> percentiles and range.



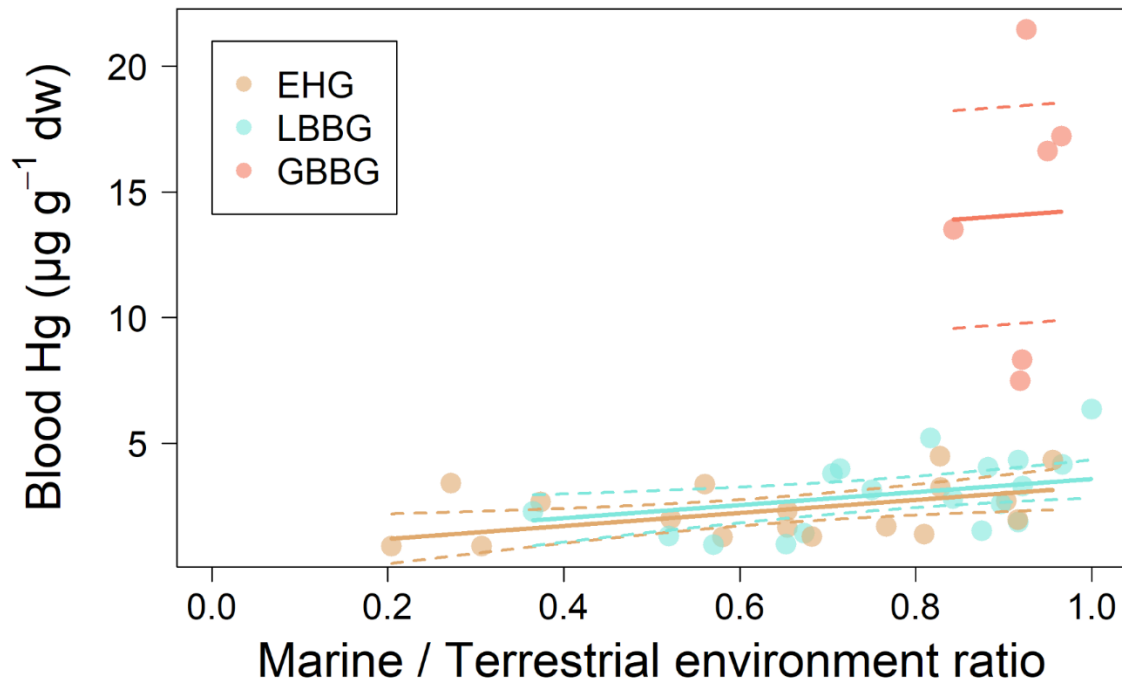
**Figure 3.** Relationship between blood Hg concentrations and PC<sub>1</sub> ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  values in adults and  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  in chicks) or PC<sub>2</sub> ( $\delta^{34}\text{S}$  values in chicks) of four gull species from Ile de Ré, France: European herring gulls (EHG), Lesser black-backed gulls (LBBG), Great black-backed gulls (GBBG) and Yellow-legged gulls (YLG). For each species, the solid line refers to the fitted models obtained and dotted lines represent 95 % confident intervals.





535

**Figure 4.** Relationship between blood Hg concentrations and the proportion of foraging trips in marine (1: exclusively marine) vs terrestrial (0: exclusively terrestrial) habitats obtain by GPS tracking in adults of three gull species from Ile de Ré, France; European herring gulls (EHG), Lesser black-backed gulls (LBBG) and Great black-backed gulls (GBBG). For each species, the solid line refers to the fitted models obtained and dotted lines represent 95 % confident intervals.





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