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Mercury contamination and potential health risk to French seabirds: A multi-species and multi-site study

Prescillia Lemesle^{a,b,*}, Alice Carravieri^{a,b}, Gauthier Poiriez^a, Romain Batard^c, Aurélie Blanck^d, Armel Deniau^c, Gilles Faggio^e, Jérôme Fort^a, Fabrice Gallien^f, William Jouanneau^b, Gilles le Guillou^g, Carole Leray^h, Karen D. McCoyⁱ, Pascal Provost^c, Marie-Catherine Santoni^e, Manrico Sebastiano^j, Olivier Scher^k, Alain Ward^l, Olivier Chastel^b, Paco Bustamante^a

^a Littoral Environnement et Sociétés (LIENSs), UMR 7266 CNRS - La Rochelle Université, 17000 La Rochelle, France

^b Centre d'Etudes Biologiques de Chizé (CEBC), UMR 7372 CNRS - La Rochelle Université, 79360 Villiers-en-Bois, France

^c Ligue pour la Protection des Oiseaux (LPO), 17300 Rochefort, France

^d Office Français de la Biodiversité (OFB), 94300 Vincennes, France

^e Office de l'Environnement de la Corse (OEC), 20250 Corte, France

^f Groupe Ornithologique Normand (GONm), 14000 Caen, France

^g Maison de l'Estuaire, 76600 Le Havre, France

^h Tour du Valat, Research Institute for the Conservation of Mediterranean Wetlands, 13200 Arles, France

ⁱ Maladies Infectieuses et Vecteurs: Ecologie, Génétique, Evolution et Contrôle (MIVEGEC), Université de Montpellier – CNRS – IRD, 34090 Montpellier, France

^j Behavioural Ecology & Ecophysiology Group, Department of Biology, University of Antwerp, 2610 Wilrijk, Belgium

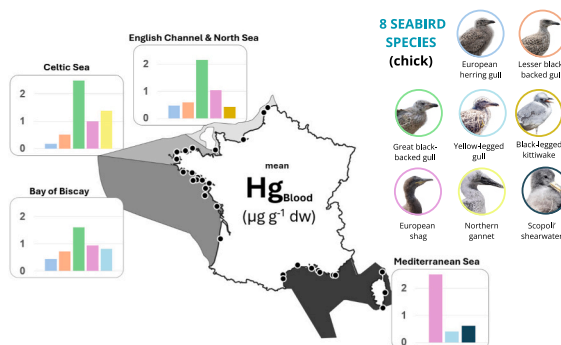
^k Conservatoire d'espaces naturels d'Occitanie (CEN Occitanie), 34000 Montpellier, France

^l Groupe ornithologique et naturaliste (GON, agrément régional Hauts-de-France), 59000 Lille, France

HIGHLIGHTS

- Blood Hg was analysed in chicks of eight seabird species from 32 sites in France.
- Influences of extrinsic and intrinsic factors on Hg concentrations were evaluated.
- Feeding ecology, especially $\delta^{15}\text{N}$ values was the main driver of Hg variation.
- No strong geographical difference in seabird Hg contamination was reported.
- According to Hg toxicity threshold, 74% of chicks were categorized as no risk.

GRAPHICAL ABSTRACT



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ABSTRACT

Mercury (Hg) is a naturally occurring highly toxic element which circulation in ecosystems has been intensified by human activities. Hg is widely distributed, and marine environments act as its main final sink. Seabirds are relevant bioindicators of marine pollution and chicks are particularly suitable for biomonitoring pollutants as they reflect contamination at short spatiotemporal scales. This study aims to quantify blood Hg contamination

* Corresponding author at: Centre d'Etudes Biologiques de Chizé (CEBC), UMR 7372 CNRS - La Rochelle Université, 79360 Villiers-en-Bois, France.
E-mail address: prescillia.lemesle@cebc.cnrs.fr (P. Lemesle).

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Chick
Stable isotopes
Metal
Atlantic
Mediterranean Sea

and identify its drivers (trophic ecology inferred from stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$), geographical location, chick age and species) in chicks of eight seabird species from 32 French sites representing four marine subregions: the English Channel and the North Sea, the Celtic Sea, the Bay of Biscay and the Western Mediterranean. Hg concentrations in blood ranged from $0.04 \mu\text{g g}^{-1}$ dry weight (dw) in herring gulls to $6.15 \mu\text{g g}^{-1}$ dw in great black-backed gulls. Trophic position ($\delta^{15}\text{N}$ values) was the main driver of interspecific differences, with species at higher trophic positions showing higher Hg concentrations. Feeding habitat ($\delta^{13}\text{C}$ values) also contributed to variation in Hg contamination, with higher concentrations in generalist species relying on pelagic habitats. Conversely, colony location was a weak contributor, suggesting a relatively uniform Hg contamination along the French coastline. Most seabirds exhibited low Hg concentrations, with 74% of individuals categorized as no risk, and $< 0.5\%$ at moderate risk, according to toxicity thresholds. However, recent work has shown physiological and fitness impairments in seabirds bearing Hg burdens considered to be safe, calling for precautionary use of toxicity thresholds, and for studies that evaluate the impact of Hg on chick development.

1. Introduction

Mercury (Hg) is an element naturally present in the environment, but anthropogenic activities have contributed to significant releases that well exceed natural emissions (Gworek et al., 2020; Streets et al., 2019). Once in the aquatic environment, Hg in its inorganic form (iHg) is transformed into methylmercury (MeHg) mainly via microbial processes (Gworek et al., 2016). In contrast to iHg, MeHg is highly bioavailable and is also very toxic for biota (Sakamoto et al., 2011). MeHg bioaccumulates in the tissues of living organisms over time and biomagnifies within food webs, a process which is particularly exacerbated in marine ecosystems due to more complex food webs and high bioconcentration in first trophic levels (Chételat et al., 2020; Lavoie et al., 2015; Liu, 2012). Furthermore, MeHg induces a wide range of adverse effects, including neurological, endocrine, behavioral, immune and reprotoxic effects, even at low concentrations (e.g., Chastel et al., 2022; Holmes et al., 2009; Kalisińska, 2019; Scheuhammer et al., 2007; Wolfe et al., 1998), with demonstrated effects on wild seabird populations (Goutte et al., 2014a, 2014b).

The Minamata Convention on Mercury was adopted to reduce anthropogenic emissions and thus the risks of Hg exposure to humans and wildlife (<https://www.mercuryconvention.org>). However, divergences between trends of Hg in marine biota and anthropogenic emissions are increasingly being reported (Lippold et al., 2020; Morris et al., 2022; Tartu et al., 2022; Wang et al., 2019), and the effectiveness of the Minamata Convention on Hg levels in marine predators is still unclear (Médiéu et al., 2024). In the marine environment, Hg is highly persistent and has a complex biogeochemical cycle that is influenced by multiple cascade processes that could explain the uncoupling between emissions and biota Hg burdens (Obrist et al., 2018; Sonke et al., 2023; Wang et al., 2019). Global change is also a key driver that could contribute to increasing Hg exposure in biota (Wang et al., 2019). Moreover, reductions in emissions are not predicted to induce strong and quick reductions of environmental concentrations, especially if there is no decisive action to reduce Hg emissions (Schartup et al., 2022). Therefore, Hg is an ongoing and future threat for marine ecosystems and a better understanding of exposure and effects on marine fauna is essential. Article 19 of the Minamata Convention on Mercury calls for increased geographical monitoring of Hg burdens in marine fauna, including marine mammals, sea turtles and seabirds, which are all good bioindicators of Hg contamination of the environment (Evers et al., 2024). In addition, in 2008, the European Union adopted the Marine Strategy Framework Directive (MSFD), a legislative text requiring European member states to assess, achieve or maintain a Good Environmental Status (GES) of their marine waters. The MSFD aims to protect the marine environment against harm caused by chemical contaminants (Descriptor 8), including Hg, and implies monitoring of concentrations in different environmental matrices, and of biological effects. Commonly monitored biota includes fish and invertebrates (notably *Mytilus* and *Crassostrea* spp.), while upper marine predators are largely overlooked (Mille et al., 2023). Contaminant biomonitoring

should be multi-sites and include species with diverse trophic ecologies and wide spatial distributions (Wang et al., 2019), but is still sparse in European waters and rarely carried out in marine predators such as seabirds, which hinders the comprehensive assessment of Hg contamination and its risks across food webs.

Seabirds, as long-lived, mid to top predators, can accumulate large Hg quantities and consequently suffer physiological and fitness impairments (reviewed in Ackerman et al., 2016; Chastel et al., 2022; Whitney and Cristol, 2017). Due to their life history characteristics, these birds are relevant spatiotemporal bioindicators of Hg contamination in coastal and oceanic ecosystems (Burger and Gochfeld, 2004; Elliott and Elliott, 2013). Adult seabirds are commonly used to assess Hg contamination through non-lethal sampling of feathers and blood (Albert et al., 2019; Peterson et al., 2019; Renedo et al., 2018). Blood is a relevant matrix to evaluate recent Hg exposure due to its relatively short half-life in this tissue (Monteiro and Furness, 2001a). However, recent works suggest that past Hg exposure during migration and/or overwintering can significantly affect circulating levels during breeding (Carravieri et al., 2023; Lavoie et al., 2015, 2014). This complicates the use of adult seabirds to evaluate local Hg contamination. Moreover, intrinsic traits such as sex (related to variability in feeding strategies and maternal transfer; Ackerman et al., 2020; Carravieri et al., 2014b; Robinson et al., 2012), breeding stage (Tartu et al., 2013; Tavares et al., 2013) and moulting patterns (Bearhop et al., 2000; Carravieri et al., 2014a) can also influence adult blood Hg concentrations. Thus, interpreting Hg concentrations of adults requires detailed knowledge on migration routes, wintering areas, and moulting strategies (Albert et al., 2019; Carravieri et al., 2023, 2014a). In pre-fledging chicks (hereafter chicks), egg-derived Hg resulting from maternal transfer is excreted in down feathers and diluted during growth (Ackerman et al., 2011). In most species, seabird chicks are fed by their parents with food obtained in areas close to the colony, representing a restricted spatiotemporal window. Chicks therefore faithfully represent recent and local Hg contamination of assimilated diet items and are commonly used in Hg monitoring using growing feathers (e.g., Blévin et al., 2013), blood (e.g., Albert et al., 2019; Carravieri et al., 2020, 2017) or both (levels in feathers and blood are tightly correlated; Binkowski et al., 2021). Species can differ in Hg contamination depending on the resources and habitats they use and/or on taxonomic differences in Hg detoxification mechanisms and efficiency (Eagles-Smith et al., 2009; Lucia et al., 2012). Multi-species studies targeting chicks may allow us to assess Hg variability between specific marine compartments and help us disentangle exposure from detoxification.

In Europe, several studies have focused on Hg contamination in seabirds over the last two decades (e.g., Albertos et al., 2020; Goutner et al., 2013; Novotna Kruzikova et al., 2023; Sánchez-Fortún et al., 2020; Sanpera et al., 2007; Szumilo-Pilarska et al., 2017; Zorrozua et al., 2020). However, studies carried out on French seabird communities have mainly targeted the overseas territories: French Guiana (six species, Sebastiano et al., 2017, 2016), Clipperton Island (six species, Bustamante et al., 2023), Reunion Island and Glorioso Archipelago (10

species, Kojadinovic et al., 2007a, 2007b, 2007c), sub-Antarctic islands and Antarctica (33 species, Blévin et al., 2013; Carravieri et al., 2020, 2018, 2017, 2014c; Cherel et al., 2018; Renedo et al., 2018). Metropolitan France hosts >225,000 breeding pairs of seabirds belonging to 28 species (GISOM, 2023), yet our knowledge about their Hg contamination is still very limited (four species in Southwestern France: Jouanneau et al., 2022; Zorrozuza et al., 2020; four species in the English Channel: Binkowski et al., 2021; Lemesle et al., 2024).

Here, we quantified Hg concentrations in the blood of chicks of eight seabird species (five Charadriiformes, two Suliformes and one Procellariiformes) from 32 sites across four marine subregions in metropolitan France (English Channel and North Sea, Celtic Sea, Bay of Biscay and Western Mediterranean Sea). The wide spatial distribution of these species on the French coasts, particularly *Laridae* species, enables multiple spatial comparisons. Moreover, this multi-species sampling represents a wide range of feeding strategies, including generalist and specialist species, using coastal and/or pelagic feeding habitats. Here, we used carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope values as proxies of feeding ecology (Bond and Jones, 2009; Lourenço et al., 2015). Specifically, $\delta^{13}\text{C}$ values indicate feeding habitats (values below -25‰ reflect a primarily terrestrial/freshwater diet, values included between -25‰ and -18‰ suggest a mix of freshwater/marine diet, and finally values above -18‰ indicate a primarily marine diet) (Bearhop et al., 1999; Hobson, 1990; Oppel and Powell, 2010) and $\delta^{15}\text{N}$ values are a proxy of trophic position, increasing by around 3.4‰ at each trophic step (Hobson et al., 1994; Post, 2002).

This large multi-species and multi-site study aimed to 1) document blood Hg concentrations; 2) determine extrinsic (site and trophic ecology) and intrinsic (chick age and species) factors driving variation in Hg concentrations; and 3) discuss potential health risks to French seabirds in relation to known Hg toxicity thresholds. Due to Hg biomagnification processes, we expected that i) species and individuals feeding in marine habitats (high $\delta^{13}\text{C}$ values) at higher trophic positions (high $\delta^{15}\text{N}$ values)

to have higher blood Hg concentrations than species feeding in terrestrial habitats (low $\delta^{13}\text{C}$ values) and feeding at lower trophic positions (low $\delta^{15}\text{N}$ values); and ii) Mediterranean populations to have the highest Hg concentrations, as previously shown in large scale studies of lower trophic level organisms on the French coastline (Briant et al., 2017; Claisse et al., 2001; Mauffret et al., 2023).

2. Materials and methods

2.1. Sample collection

Fieldwork was conducted at 32 sites along the coastline of metropolitan France (including Corsica) in the summers of 2019, 2020 and 2021 during the chick-rearing period (Fig. 1). Twenty sites were sampled in three marine subregions of the Atlantic coast (English Channel and North Sea, Celtic Sea and Bay of Biscay) as well as 12 sites along the Mediterranean coast (Western Mediterranean marine subregion). Eight species were sampled: five *Laridae* species (European herring gulls *Larus argentatus argentatus*, lesser black-backed gulls *L. fuscus graellsii*, great black-backed gulls *L. marinus*, yellow-legged gulls *L. michahellis*, and black-legged kittiwakes *Rissa tridactyla* (“herring gulls”, “lesser b-b gulls”, “great b-b gulls”, “yellow-l. gulls” and “kittiwakes”, respectively, hereafter)), European shags *Gulosus aristotelis* (“shags” hereafter), northern gannets *Morus bassanus* (“gannets” hereafter) and Scopoli’s shearwaters *Calonectris diomedea* (“shearwaters” hereafter). Adults of these eight species forage in areas close to the colony to feed their chicks but provide diverse diets (*i.e.*, individual birds can be specialists or generalists) from different habitats (terrestrial, coastal, oceanic, benthic or/and epipelagic environments) depending on species (Table S1). In total, 849 well-feathered chicks were captured before fledging, measured, blood sampled and ringed ($n = 2\text{--}38$ individuals per species and site), except for gannet chicks, which were caught just after fledging using a dip net from a boat. All other species

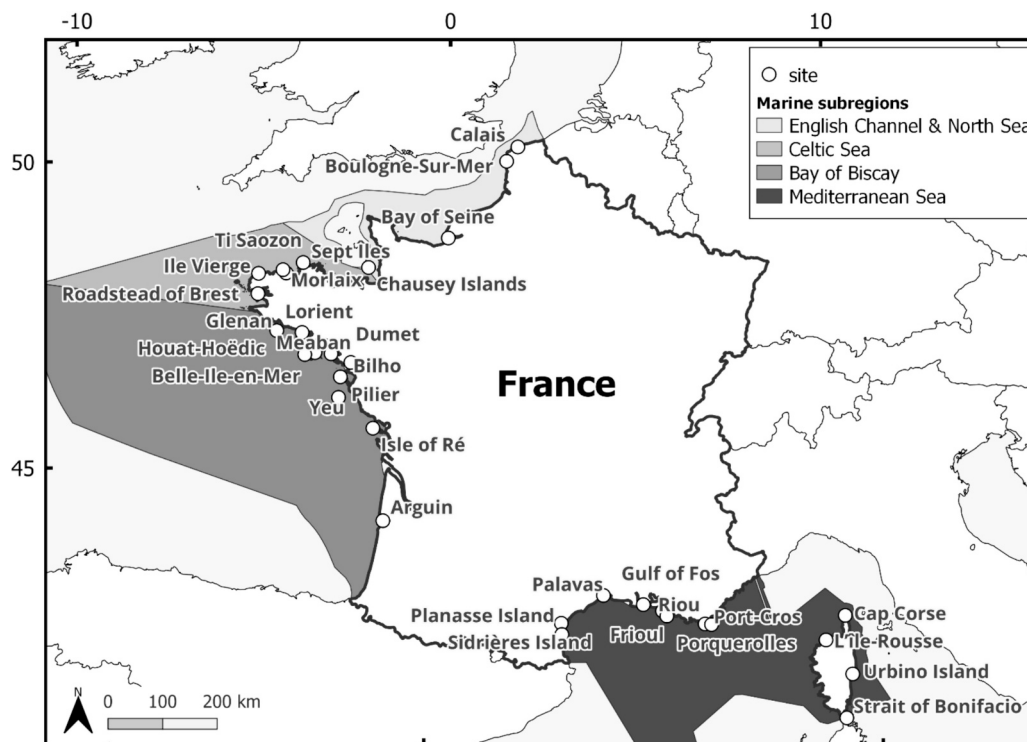


Fig. 1. Sampling sites in different marine subregions of the metropolitan French coast. Circles represent sampling colonies. Marine subregions are represented by different shades of grey.

were caught by hand around or in the nest (shags, *Laridae* species) or burrow (shearwaters). Biometric measurements were taken using a Pesola spring balance (body mass), a sliding calliper with a 0.1 mm accuracy for skull (head + bill), beak (thickness and length) and tarsus length, and a ruler with a 1 mm accuracy was used for wing length. Blood (2–3 ml depending on the species size, <1% of the body mass) was collected with heparinized syringes and 23 or 25-gauge needles from the brachial vein. Blood samples were transferred into Eppendorf® tubes and stored in a cool box during fieldwork. Plasma and red blood cells (RBC) were separated by centrifugation at 4000 rpm for 6 min at 4 °C

within 2 to 6 h after sampling. Where possible, RBC and plasma samples were stored in liquid nitrogen until their final storage in a freezer (−20 °C for RBC).

2.2. Mercury analyses

Due to the relatively short half-life of Hg in blood (estimated to be less than a week for chicks of Cory's shearwater *Calonectris borealis*, Monteiro and Furness, 2001b), blood is commonly used to quantify Hg concentrations over a recent temporal scale (Albert et al., 2019). In

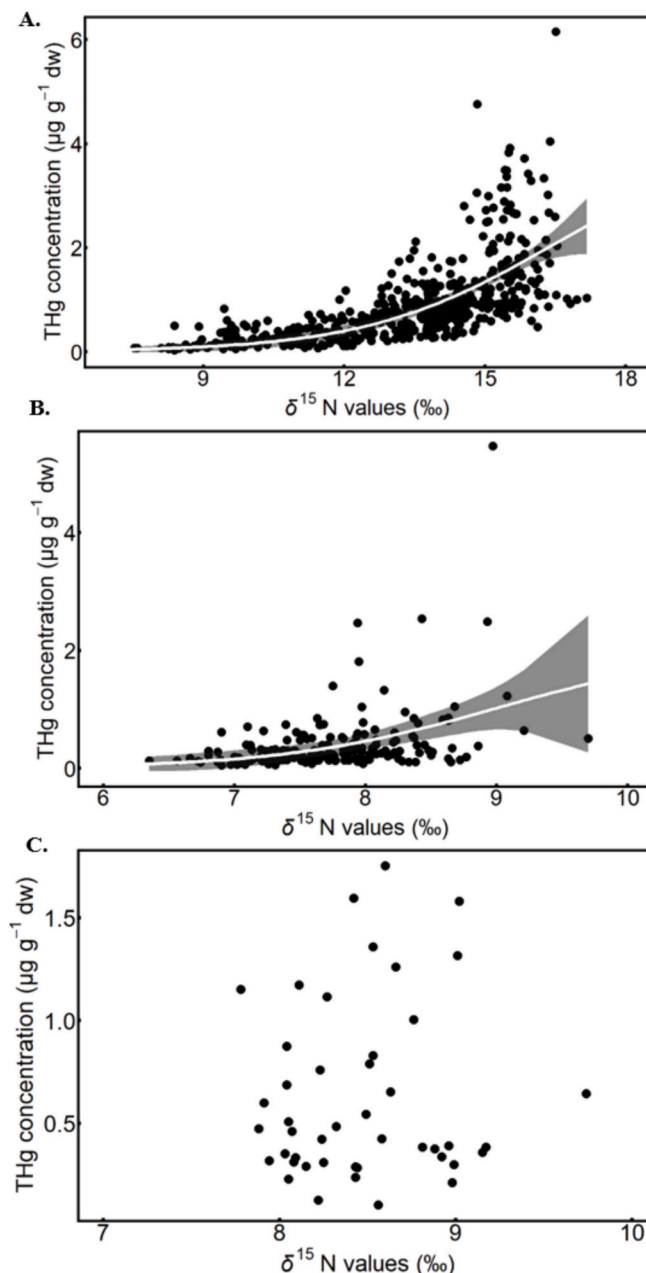


Fig. 2. Mercury concentrations ($\mu\text{g g}^{-1} \text{dw}$) association with $\delta^{15}\text{N}$ values (‰) for each dataset (A. Atlantic, B. Mediterranean yellow-legged gull, C. Mediterranean Scopoli's shearwater). The white line represents a fitted non-linear logistic equation $y = \frac{\text{asym}}{1 + e^{\left(\frac{x - \text{mid}}{\text{scal}}\right)}}$ with 95% confidence interval in grey.

Estimated parameters are $\beta \pm \text{SE}$: A. *asym* 4.08 ± 1.54 , *xmid* 16.42 ± 1.40 and *scal* 2.00 ± 0.33 and $\Delta\text{AICc}_{\text{nls-null}} = 348$ for the Atlantic dataset and B. *asym* 2.03 ± 1.63 , *xmid* 9.00 ± 1.24 and *scal* 0.81 ± 0.32 and $\Delta\text{AICc}_{\text{nls-null}} = 27$ for the Mediterranean yellow-legged gull dataset.

blood, total Hg concentrations are a close approximation of MeHg concentrations (~100%, [Renado et al., 2021](#)). Furthermore, Hg in blood is mainly associated with the cellular fraction ([Bond and Robertson, 2015](#); [Renado et al., 2018](#)). We will thus use the term “blood” to refer to RBC hereafter, unless otherwise specified. Blood was freeze-dried and homogenised to powder and Hg was quantified in subsamples of this powder (mean \pm SD, 1.38 ± 0.72 mg dry weight (dw)) using an Advanced Mercury Analyser (@Altec AMA 254 spectrophotometer) at the LIENSs laboratory. Hg was quantified in duplicate or triplicate ($n = 1705$ subsamples from 849 individuals) such that the relative standard deviation was below 10% (mean \pm SD: $1.67 \pm 0.02\%$). The retained concentration is the mean value of replicate measurements. Two certified reference materials for trace elements were analysed under the same conditions of the samples: DOLT-5 (dogfish liver, Hg-certified value: $0.44 \pm 0.18 \mu\text{g g}^{-1}$ dw from National Research Council of Canada (NRCC), [Yang et al., 2014](#)) or TORT-3 (lobster hepatopancreas, Hg-certified value: $0.29 \pm 0.02 \mu\text{g g}^{-1}$ dw from NRCC, [Willie et al., 2013](#)). Recovery rates (\pm SD) were $98.3 \pm 0.90\%$ (DOLT-5) and $103.3 \pm 4.3\%$ (TORT-3). Blanks were measured before each run and the limit of detection of the AMA was 0.1 ng. Hg concentrations are expressed in $\mu\text{g g}^{-1}$ dw.

2.3. Stable isotope analyses

Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values have a rapid turnover in blood, up to one month, providing short-term insights on trophic ecology ([Bearhop et al., 2002](#); [Lourenço et al., 2015](#); [Ogden et al., 2004](#); [Vander Zanden et al., 2015](#)). Stable isotope analyses were carried out at the LIENSs laboratory. The relative abundance of carbon and nitrogen stable isotopes were measured from subsamples of blood powder (0.36 ± 0.09 mg, $n = 849$) packed into tin containers. Isotopic percentages were quantified with a continuous flow mass spectrometer (Thermo Scientific Delta V Plus®) coupled to an elemental analyser (Thermo Scientific EA Flash®). Isotopic data were defined by the following equation: $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ (‰) = $((R_{\text{sample}}/R_{\text{standard}}) - 1) \times 1000$ where R represents the $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ ratio. Isotopic values were expressed as standard delta (δ) notations based on international standards, Vienna PeeDee Belemnite and atmospheric nitrogen (N_2) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. Three certified reference materials (USGS-61, USGS-62 and USGS-63, caffeine) were analysed to check the accuracy ([Schimmelmann et al., 2016](#)). The analytical precision was <0.10 ‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values.

2.4. Statistical analyses

All analyses and data representation were carried out using R (version 4.2.2., [R Core Team, 2020](#)). Species sampled at only one site

(kittiwakes and gannets), as well as data from sites with less than five sampled individuals (*i.e.*, lesser b-b gulls from Morlaix, great b-b gulls from Yeu and yellow-l. gulls from Pietra Island) were used only for descriptive statistics, but were excluded from regression analyses (Table S2). Six individuals presented very low $\delta^{13}\text{C}$ values (herring gull: -28.60 ‰ and great b-b gulls: -28.25 ‰, -26.65 ‰, -26.54 ‰, -26.47 ‰ from the same sampling site, Bilho, and one yellow-l. gull: -24.03 ‰). These values were unusually low compared to all other individuals (range, for all other samples from the Atlantic: $[-24.90; -16.95]$ and for other yellow l. gulls from the Atlantic: $[-19.37; -17.42]$; Table S3), and could be explained by a different food source, particularly at the Bilho site (a sandbank inside the Loire estuary allowing the gulls to feed on freshwater and/or terrestrial prey). As the aim of the study was to identify the main drivers on a large scale, these values were excluded from the dataset. In addition, due to strong differences in isotopic baselines ([Graham et al., 2010](#)), data from the Atlantic and the Mediterranean coasts were analysed separately. The “Atlantic dataset” included three marine subregions (English Channel and North Sea, Celtic Sea and Bay of Biscay) and five seabird species (shags and four *Laridae* species: herring gulls, lesser b-b gulls, great b-b gulls and yellow-l. gulls). Two species (yellow-l. gull and shearwater) were sampled in the Mediterranean, and their datasets were analysed separately from each other (“Mediterranean yellow-l. gull dataset” and “shearwater dataset”) because of non-overlapping of $\delta^{13}\text{C}$ values (range, yellow-l. gulls: $[-19.64; -18.64]$ ‰ and shearwaters: $[-24.8; -19.75]$ ‰, Table S3), due to strong differences in feeding habitat (coastal/terrestrial vs. oceanic, respectively, see Table S1).

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were strongly and positively associated (Figs. S1 and S2) in the Atlantic dataset and in the Mediterranean yellow-l. gull dataset. Preliminary analyses showed that $\delta^{15}\text{N}$ values were a strong predictor of Hg concentrations in both datasets. We were also interested in the effect of feeding habitat ($\delta^{13}\text{C}$ values) on blood Hg concentrations but could not include $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the same model because of their collinearity. We thus fitted a non-linear logistic model (nlm with SSlogis function from “stats” package) with Hg concentrations as the response variable and $\delta^{15}\text{N}$ values as the explanatory variable (Fig. 2), and then extracted residuals from these nlm models (named “Hg partial residuals” hereafter), to use them as the response variable in multifactorial regressions. Using partial residuals was an approach already applied to deal with collinearity between two predictors (*e.g.*, [Bond and Diamond, 2009](#); [Médiéu et al., 2022](#); [Ricca et al., 2008](#); [Tartu et al., 2022](#)). This approach enabled us to quantify the effects of site, feeding habitat ($\delta^{13}\text{C}$ values), and intrinsic factors (species and chick age, inferred from body mass) on the residual variation in Hg concentrations, after accounting for the effect of trophic position ($\delta^{15}\text{N}$ values).

Table 1

Model selection of linear mixed-effects models (LMEs) for the relationship between Hg partial residuals and extrinsic ($\delta^{13}\text{C}$ values and marine subregion) and intrinsic (chick mass and species) variables for the Atlantic dataset (including herring gull, lesser black-backed gull, great black-backed gull, yellow-legged gull, and European shag), based on Akaike's Information Criterion corrected for small sample sizes (AICc). Only the first ten models and the null model are presented ($n_{\text{total}} = 20$). The most parsimonious model is given in bold. K: number of parameters; ΔAICc : difference between the model with the smallest AICc-value and the model of interest; AICcwt: Akaike's weight.

LMEs with site as a random factor and an exponential variance weight structure ($\delta^{13}\text{C}$ differed per species), $n = 554$									
Models	$\delta^{13}\text{C}$	Species	Mass	Marine subregion	$\delta^{13}\text{C}$:species	K	AICc	ΔAICc	AICcwt
Mod1	X	X	X	X	X	20	350.9	0	0.387
Mod2	X	X		X	X	19	350.9	0.06	0.376
Mod3	X	X			X	17	353.1	2.29	0.123
Mod4	X	X	X		X	18	353.3	2.44	0.114
Mod5	X	X	X	X		16	574.6	223.76	0
Mod6	X	X		X		15	575.2	224.38	0
Mod 7	X		X	X		12	577.2	226.35	0
Mod 8	X	X	X			14	579	228.14	0
Mod 9	X	X				13	579.4	228.58	0
Mod 10	X		X			10	582.6	231.71	0
Null model						8	592.5	241.63	0

For the response “Hg partial residual”, we built linear mixed-effects models (LMEs, package “MuMin”, Bartoń, 2022), with site as a random variable for both the Atlantic and Mediterranean yellow-l. gull datasets. For the Atlantic dataset, mass (continuous variable) and $\delta^{13}\text{C}$ (continuous variable), species (categorical variable), marine subregions (categorical variable), and the $\delta^{13}\text{C}$ -species interaction were the fixed explanatory variables. For the Mediterranean yellow-l. gull dataset, LMEs included only mass and $\delta^{13}\text{C}$ values as explanatory variables. For both datasets, continuous variables (mass and $\delta^{13}\text{C}$ variables) were scaled (mean = 0, SD = 1) to facilitate the comparison of effect sizes. In addition, to deal with residuals heteroscedasticity, exponential variance weights (package “nlme”, Pinheiro et al., 2023) were added to the LMEs of both datasets (the exponential variance weight structure depended on $\delta^{13}\text{C}$ values and species in the Atlantic dataset, and only on $\delta^{13}\text{C}$ values for the Mediterranean yellow-l. gull dataset). For the Atlantic dataset, pairwise comparisons with post-hoc Tukey tests were performed to evaluate differences in Hg partial residuals between marine subregions and between species (“emmeans” package, Lenth et al., 2023).

In the shearwater dataset, $\delta^{13}\text{C}$ values were negatively correlated with $\delta^{15}\text{N}$ values (see details in Fig. S1), and Hg concentrations were not significantly associated to $\delta^{15}\text{N}$ values (Fig. 2). Therefore, LMEs were constructed with Hg concentration as the response variable, mass and $\delta^{13}\text{C}$ values as fixed effects, and site as a random effect. Mass and $\delta^{13}\text{C}$ values were scaled (mean = 0, SD = 1).

For all the models, body mass was used as a proxy of chick age, as shown in several seabird species (e.g., Barrett, 1996; Lequette and

Weimerskirch, 1990; Nelson, 1964); tarsus length was also tested as a proxy of chick age and led to the same results when used in regression models (data not shown).

For each initial model, the homoscedasticity and normal distribution of residuals were visually assessed (residuals *versus* fitted values, Q-Q graphs, Zuur et al., 2009). We performed model selection using the Akaike information criterion for small samples (AICc, “MuMin” package, Bartoń, 2022) to identify the best model(s). Explanatory variables included in the best model(s) were considered to have a significant effect, if the focal model performed better than the null model and other alternative models. If the difference in AICc values between two or more models (ΔAICc) was >2 , the model with the lowest AICc was considered as the best. In contrast, if $\Delta\text{AICc} < 2$, models were considered of equal explanatory power. The explained deviance was calculated compared to the null model (Zuur et al., 2009). The relative importance of predictor variables was calculated by summing AICc_{wt} (sum of weight, SW) of models including this variable. A significance level of $\alpha < 0.05$ was used for post-hoc Tukey tests. Result of LMEs were graphically presented using estimated marginal means with the “emmeans” package.

3. Results

3.1. Mercury concentrations in blood

Along the Atlantic coast, the lowest mean Hg concentrations were reported in herring gulls, while the highest were reported in great b-b

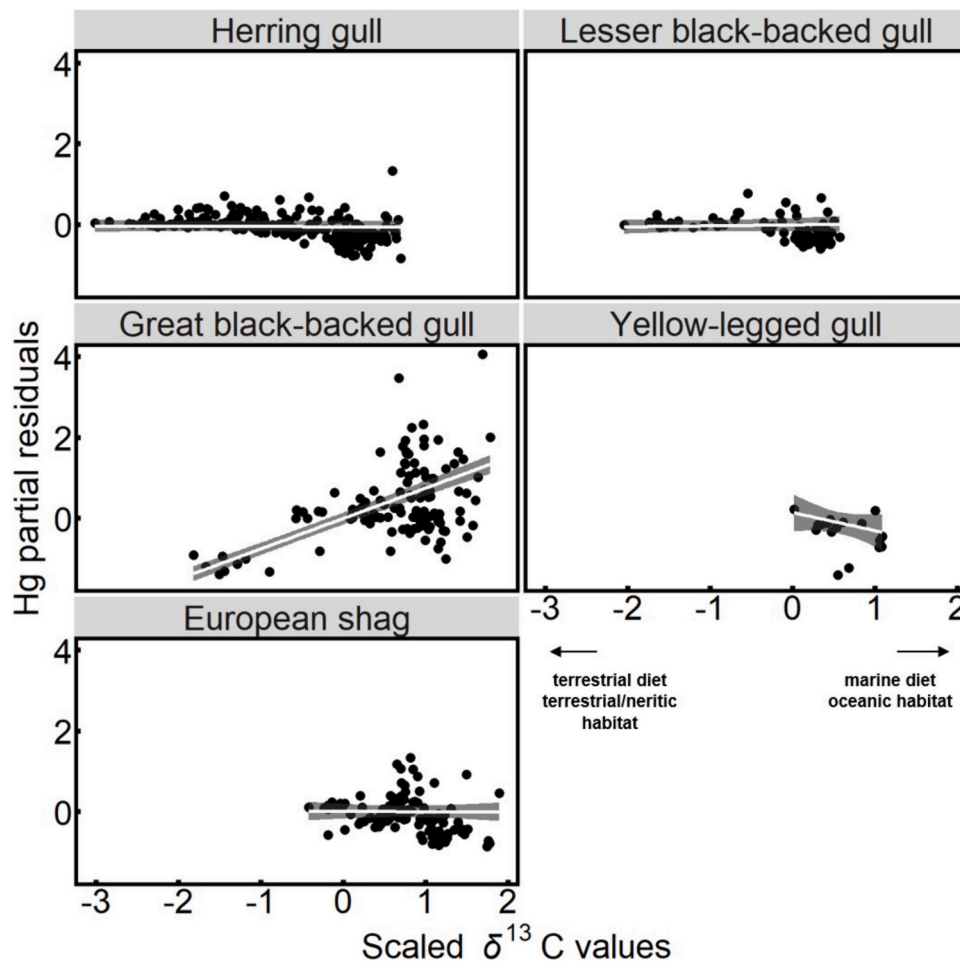


Fig. 3. Associations between Hg partial residuals and scaled $\delta^{13}\text{C}$ values in chicks of different seabird species from the French Atlantic coastline. The solid line represents the species-specific fitted models, and 95% confident interval is presented in grey. Six outliers were removed from the statistical analysis (see M&M).

gulls. In the English Channel and North Sea, mean blood Hg concentrations ranged from 0.33 ± 0.32 in herring gulls to $2.86 \pm 0.85 \mu\text{g g}^{-1}$ dw in great b-b gulls. Similarly, Hg concentrations in the Celtic Sea ranged from 0.11 ± 0.05 in herring gulls to $2.86 \pm 1.31 \mu\text{g g}^{-1}$ dw in great b-b gulls and in the Bay of Biscay from 0.27 ± 0.13 to $1.95 \pm 0.58 \mu\text{g g}^{-1}$ dw in herring gulls and great b-b gulls, respectively. Finally, in the Mediterranean Sea, Hg concentrations ranged from 0.24 ± 0.13 in yellow-l. gulls to $2.99 \pm 0.59 \mu\text{g g}^{-1}$ dw in shags. For each species, the mean (\pm SD), median and range of Hg concentrations, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, for each sample site are presented in the SI (Tables S2, S3 and S4).

3.2. Extrinsic and intrinsic drivers of Hg concentrations

For the Atlantic dataset, ranges of partial Hg residuals were $[-0.84; 1.34]$ in herring gulls, $[-0.59; 0.77]$ for lesser b-b gulls, $[-1.38; 4.06]$ for great b-b gulls, $[-1.41; 0.22]$ for yellow-l. gulls and $[-0.86; 1.34]$ for shags. The best model explaining blood Hg partial residuals included all variables (scaled $\delta^{13}\text{C}$ values, species, and their interaction, and marine subregion), except mass, and explained 46% of the total variation (Table 1). Hg partial residuals were negatively associated with scaled $\delta^{13}\text{C}$ values, but the effect size was small (LME; estimate \pm SE, -0.01 ± 0.02 ; $F_{1,525} = 35.42$), and the association depended on species (post-hoc Tukey test, p -value < 0.01 , Table S5). Specifically, great b-b gulls had a positive association between Hg partial residuals and scaled $\delta^{13}\text{C}$ values (LME; 0.75 ± 0.04 , Fig. 3), while other species had a weak negative association (small effect sizes; -0.44 ± 0.32 in yellow-l. gulls, -0.01 ± 0.74 in shags, -0.01 ± 0.02 in herring gulls and 0.03 ± 0.04 in lesser b-b gulls). Post-hoc comparisons of Hg partial residuals between species, and between marine subregions showed no significant differences (post-hoc Tukey tests, all p -values > 0.05 , Table S5, Fig. S3). For the Mediterranean yellow-l. gull dataset, $\delta^{13}\text{C}$ values were a significant predictor of Hg partial residuals with a weak positive association (LME; 0.07 ± 0.03 ; $F_{1,169} = 6.34$, Fig. 4 and Table S6). For the shearwater dataset, models including scaled $\delta^{13}\text{C}$ values alone, or scaled $\delta^{13}\text{C}$ values and mass, and the null model all had similar explanatory power ($\Delta\text{AICc} < 2$; Table S6).

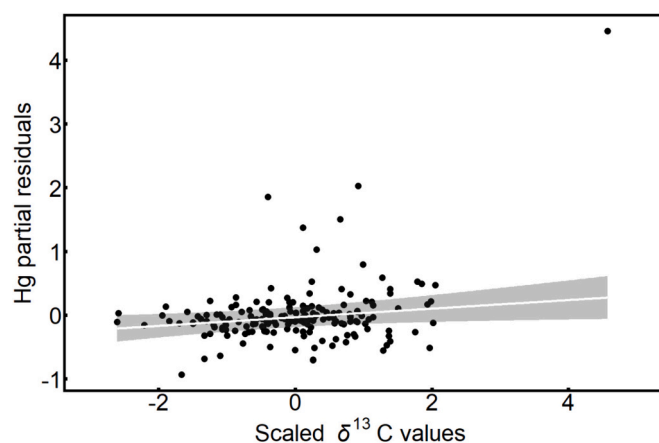


Fig. 4. Association between Hg partial residuals and scaled $\delta^{13}\text{C}$ values for yellow-legged gull chicks from the French Mediterranean region. The solid line represents the fitted model and 95% confident interval is presented in grey. $\beta \pm$ SE: slope 0.07 ± 0.03 , intercept -0.03 ± 0.07 , $n = 180$, linear mixed-effects model with site as a random factor, $\Delta\text{AICc}_{\text{model-null}} = 4$).

4. Discussion

This study is the first description of Hg concentrations in the blood of chicks of eight seabird species along the coast of metropolitan France. Our large scale, multi-site, and multi-species study showed no strong geographical difference in seabird Hg contamination among Atlantic marine subregions (English Channel and North Sea, Celtic Sea and Bay of Biscay), nor between the Atlantic and Mediterranean coasts. Trophic ecology (feeding habitat and trophic position inferred from $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values) was identified as the main driver of variation in Hg concentrations, which were higher in species feeding in marine habitats at higher trophic positions.

4.1. Mercury concentrations in blood: comparison among seabirds

Hg concentrations were consistent with values previously reported in seabird chicks in metropolitan France (Bay of the Seine River, Chausey Islands, Isle of Ré, Binkowski et al., 2021; Jouanneau et al., 2022; Table S7). However, Hg concentrations in herring gulls from the Chausey Islands were higher than those reported in 2015–2018 (Binkowski et al., 2021), while isotopic values (mean \pm SD: $\delta^{13}\text{C}$: $-20.60 \pm 1.22 \text{‰}$ and $\delta^{15}\text{N}$: $11.74 \pm 1.56 \text{‰}$) were comparable, suggesting that other factors might explain the higher Hg concentrations reported here (Tables S3 and S7). Mean Hg concentrations were overall within the range of those documented previously in chicks of other seabird species and sites (e.g., Southern Ocean, Western Atlantic Ocean, Pacific Ocean; Table S7). Herring gulls, yellow-l. gulls and kittiwakes showed the lowest concentrations in our dataset ($< 0.5 \mu\text{g g}^{-1}$ dw) and were comparable to those of other gull species from the Western Atlantic Ocean or Pacific Ocean (Table S5; Goodale et al., 2008; Peterson et al., 2017; Sebastiano et al., 2017). Lesser b-b gulls and shearwaters had intermediate Hg concentrations ($\sim 0.6 \mu\text{g g}^{-1}$ dw), within the same range as south polar skua chicks *Catharacta maccormicki* and penguin chicks *Pygoscelis* sp. (Antarctica, Carravieri et al., 2017; Souza et al., 2020). Scopoli's shearwaters had Hg concentrations comparable to chicks of the same species from Malta, in the southern eastern Mediterranean (0.6 to $1.0 \mu\text{g g}^{-1}$ dw, Costantini et al., 2020). Shags, gannets and great b-b gulls had the highest concentrations (mostly $> 1 \mu\text{g g}^{-1}$ dw), comparable to seabird chicks from French Guiana (magnificent frigatebirds *Fregata magnificens*), Canada (Glaucous gulls *Larus hyperboreus*), USA (Forster's terns *Sterna forsteri*), Crozet Islands (brown skuas *Catharacta skua lönnbergi*) and Kerguelen Islands (white-chinned petrels *Procellaria aequinoctialis*) (Burnham et al., 2018; Carravieri et al., 2020, 2017; Eagles-Smith et al., 2008; Sebastiano et al., 2016).

At the individual level, some birds had relatively high Hg concentrations, while their isotopic values were within the same range of other individuals. This suggests potential point contamination of the food supplied to the chicks. In particular, one yellow-l. gull chick ($5.46 \mu\text{g g}^{-1}$ dw) had Hg concentrations comparable to the adults (mean adult yellow-l. gulls: $6.00 \pm 2.72 \mu\text{g g}^{-1}$ dw, Jouanneau et al., 2022) and one great b-b gull chick ($6.2 \mu\text{g g}^{-1}$ dw) had a concentration higher than the maximum Hg concentration currently reported in the blood of seabird chicks (brown skua: $5.8 \mu\text{g g}^{-1}$ dw in Carravieri et al., 2017).

4.2. Intrinsic and extrinsic drivers of Hg concentrations

4.2.1. Mercury and feeding ecology

Variation in Hg concentrations at individual, species and community levels in seabirds is often driven by trophic ecology (e.g., Ackerman et al., 2016; Binkowski et al., 2021; Carravieri et al., 2020; Chételat et al., 2020; Jouanneau et al., 2022; Sebastiano et al., 2017; Wiener et al., 2003). Blood Hg concentrations and $\delta^{15}\text{N}$ values were strongly associated in the Atlantic seabird community, consistent with the biomagnification of Hg along food webs (Lavoie et al., 2010). Species with a higher trophic position (discriminated with $\Delta\delta^{15}\text{N}$ calculated between species > 3.4 , as described by Hobson et al., 1994; Tables S3–S4–S8) had

higher Hg concentrations (great b-b gull) than species at lower trophic positions (herring gull). This positive association has already been reported in the blood of chicks from subtropical and Arctic avian communities (Burnham et al., 2018; Sebastiano et al., 2017), and in *Laridae* colonies in France (Binkowski et al., 2021; Jouanneau et al., 2022). For the Mediterranean region, a similar Hg- $\delta^{15}\text{N}$ trend was observed between individuals of yellow-l. gulls, as previously reported for chicks of other Mediterranean gull species (Audouin's gull *Larus audouinii*, Sanpera et al., 2007). No relationship between Hg concentrations and $\delta^{15}\text{N}$ values was observed in shearwaters, contrary to previous results for the same species (Costantini et al., 2020). This could be linked to the smaller range of Hg concentrations in the shearwaters of our study (0.10–1.17 $\mu\text{g g}^{-1}$ dw) compared to Costantini et al. (2020) (0.23–4.29 $\mu\text{g g}^{-1}$ dw).

Feeding habitat (marine vs. terrestrial/neritic or pelagic vs. benthic, inferred from $\delta^{13}\text{C}$ values) has previously been identified as a driver of Hg contamination in seabirds from different communities (Binkowski et al., 2021; Burnham et al., 2018; Carravieri et al., 2020; Chételat et al., 2020). Here, piscivorous specialists (shags) and opportunistic generalists (herring gulls, lesser b-b gulls and yellow-l. gulls) using neritic and/or terrestrial habitats all showed a weak negative association between Hg partial residuals and $\delta^{13}\text{C}$ values. The small effect sizes of these associations challenge their biological meaning. In contrast, great b-b gulls showed a strong positive relationship between Hg partial residuals and $\delta^{13}\text{C}$ values, as previously shown in chicks from an Arctic seabird community (Bond and Diamond, 2009) or between Hg and $\delta^{13}\text{C}$ values in gulls in France (Seine estuary, English Channel coast, Binkowski et al., 2021; Isle of Ré, Atlantic coast, Jouanneau et al., 2022). Species relying on marine resources have been shown to have higher Hg concentrations than species associated with terrestrial or coastal resources (Ackerman et al., 2016; Ochoa-acuña et al., 2002; Thorne et al., 2021). This unexpected positive Hg- $\delta^{13}\text{C}$ association (after accounting for $\delta^{15}\text{N}$) for great b-b gulls compared to the overall model could be explained by high between-individual variation in feeding strategies of great b-b gulls and an overrepresentation of terrestrial/coastal species (shags, herring gulls, lesser b-b gulls and yellow-l. gulls) compared to coastal/oceanic species (great b-b gulls), which limits our power to quantify relationships in oceanic habitats. In contrast to other gulls, the diet of great b-b gulls relies mainly on marine resources (Binkowski et al., 2021; Jouanneau et al., 2022). Unlike coastal and benthic feeders such as shags, great b-b gulls have a more opportunistic and diverse array of marine prey with potentially variable Hg content, which may lead to greater variability in Hg concentrations.

Yellow-l. gulls showed between-region differences in the association of Hg partial residuals and $\delta^{13}\text{C}$ values, with a negative and a positive correlation for the Atlantic and Mediterranean regions, respectively. However, the effect size of both relationships was small and the limited sample size in the Atlantic ($n = 20$) compared with the Mediterranean ($n = 195$) could drive divergent trends. Moreover, yellow-l. gulls from the Mediterranean Sea showed a larger variation in $\delta^{13}\text{C}$ values (coefficient of variation: Atlantic: 2.7% and Mediterranean: 7.5%) that could suggest differences in the use of marine resources depending on the region. In the Mediterranean, yellow-l. gull is reported as a generalist species with varying individual specialization in feeding habitat (e.g., diverse marine resources, urban prey, garbage and agriculture habitats) (Navarro et al., 2017; Ramos et al., 2009). These between- and within-species variations are representative of the complexity of the relationship between Hg contamination and feeding habitat (inferred from $\delta^{13}\text{C}$ values).

Shearwaters use only oceanic feeding habitats, resulting in a restricted trophic niche (Afán et al., 2014) with little isotopic variation. Accordingly, $\delta^{13}\text{C}$ values and Hg concentrations were not correlated, as previously shown in the Mediterranean for this species (Costantini et al., 2020). Using $\delta^{13}\text{C}$ values as a spatial proxy in species specialised in a single feeding habitat may be insufficient to establish the relationship between Hg concentrations and feeding habitat.

4.2.2. Mercury and spatial variability

There was no clear spatial association of Hg concentrations with marine subregions on the Atlantic coast, with each species presenting a distinct pattern (Fig. S3). For the two species with widespread sampling, herring gulls had Hg means ranging from 0.11 $\mu\text{g g}^{-1}$ dw at the Sept-Iles (Celtic Sea) to 1.74 $\mu\text{g g}^{-1}$ dw at Isle of Ré (Bay of Biscay), whereas great b-b gulls had their lowest mean Hg concentration of 1.13 $\mu\text{g g}^{-1}$ dw at Houat-Hoëdic (Bay of Biscay) and their highest concentrations of 2.86 $\mu\text{g g}^{-1}$ dw at Chausey and Brest (Celtic Sea) (Table S2). For species breeding on Atlantic and Mediterranean sites, such as yellow-l. gulls, Hg contamination was homogeneous, except for a few sites with high Hg concentrations (Isle of Ré – Bay of Biscay, the Western French Mediterranean coast, and Bonifacio – Southern Corsica; Table S2). Similarly, shags showed large site-specific variation in Hg burdens with concentrations three times higher in Bonifacio (2.99 $\mu\text{g g}^{-1}$ dw at Bonifacio – Mediterranean Sea) than in the Atlantic (ranging from 0.71 to 1.12 $\mu\text{g g}^{-1}$ dw at the Glénan Islands and Houat-Hoëdic – Bay of Biscay, respectively; Table S2). These “hotspots” of Hg contamination along the French Atlantic and Mediterranean coasts are consistent with results in lower trophic position species (Briant et al., 2017; Cresson et al., 2015). Specifically, Hg concentrations in bivalves and fish were high close to estuarine sites on the Atlantic coasts (i.e., Seine, Loire and Gironde estuaries, Briant et al., 2017) and higher on the Eastern Mediterranean coast (especially Southern Corsica) than the Western Mediterranean coasts (Briant et al., 2017; Cresson et al., 2015). Hg bioavailability is the result of complex local biogeochemical, hydrological, and topographical variabilities, which are then transferred along the food web (Driscoll et al., 2013; Kotnik et al., 2007; Mason et al., 2012; Ullrich et al., 2001). Specifically, oligotrophy was previously reported to be a main driver of Hg bioaccumulation (Chouvelon et al., 2018). Aquatic organisms of oligotrophic waters concentrate more Hg than in other types of waters, because Hg methylation rate is more efficient inducing higher Hg bioavailability, as well as a bioconcentration accentuated by the low productivity of the ecosystem, with a lower dilution of Hg among phytoplanktonic cells (Heimbürger et al., 2010; Pickhardt et al., 2002). The “Mediterranean Mercury Anomaly”, driven by oligotrophy and other factors, results in higher Hg concentrations in different taxa (crustaceans, sharks, and fish) in Mediterranean compared to Atlantic waters (Chouvelon et al., 2018; Cresson et al., 2015). However, spatial comparisons need to be considered with caution as isotopic baselines also largely differ among these zones (Chouvelon et al., 2018). Anthropogenic activities are thought to contribute to high local Hg contamination in biota inducing “hotspots” associated with proximity to cities, industrialized ports and large continental fluvial inputs (Binkowski et al., 2021; Briant et al., 2017; Cresson et al., 2015; Pereira et al., 2019). Overall, blood Hg contamination in the seabirds studied here was spatially uniform along the French coast, with only isolated sites presenting high concentrations, probably because of local geological characteristics and/or anthropogenic activities.

4.2.3. Mercury and intrinsic factors: mass and taxonomy

Age is an essential factor to consider when investigating chick exposure, as Hg bioavailability varies quadratically from hatching to the post-fledging phase (Ackerman et al., 2011). After hatching, Hg from maternal transfer and food intake is diluted by mass gain and deposited into down and later feathers (Wenzel et al., 1996). Progressively, growth and feather development cannot compensate dietary Hg intake, and blood Hg concentrations increase again (Ackerman et al., 2011). Here, body mass was used as a proxy of chick age (body mass and chick age are highly correlated in seabirds; Barrett, 1996; Lequette and Weimerskirch, 1990; Nelson, 1964), but was not a good predictor of variation in blood Hg (after accounting for $\delta^{15}\text{N}$), likely because all chicks were sampled after the dilution phase, when trophic ecology plays a prominent role in Hg concentration variation.

Interspecific differences in Hg concentrations (raw or corrected for $\delta^{15}\text{N}$) were observed within the same taxonomic groups, such as the

Larus genus with lower concentrations in herring gulls and yellow-l. gulls than in lesser b-b gulls or great b-b gulls. Compared with trophic ecology, taxonomy seems to play a minor role in interspecific differences in blood Hg concentrations, as previously reported in other seabirds at the levels of genus, family, and order (Anderson et al., 2009; Carravieri et al., 2014c).

4.3. Potential health risk of Hg for French seabirds

Based on Hg toxicity benchmarks established for whole blood of adults (Ackerman et al., 2016), most chicks (74%, $n_{no\ risk} = 623$, $n_{total} = 843$) were classified at no risk category ($<0.95\ \mu\text{g g}^{-1}\ \text{dw}$, converted from $0.2\ \mu\text{g g}^{-1}\ \text{ww}$ with moisture percentage of 79%, Eagles-Smith et al., 2008; Fig. 5) whereas 26% ($n_{low\ risk} = 216$) were classified at low

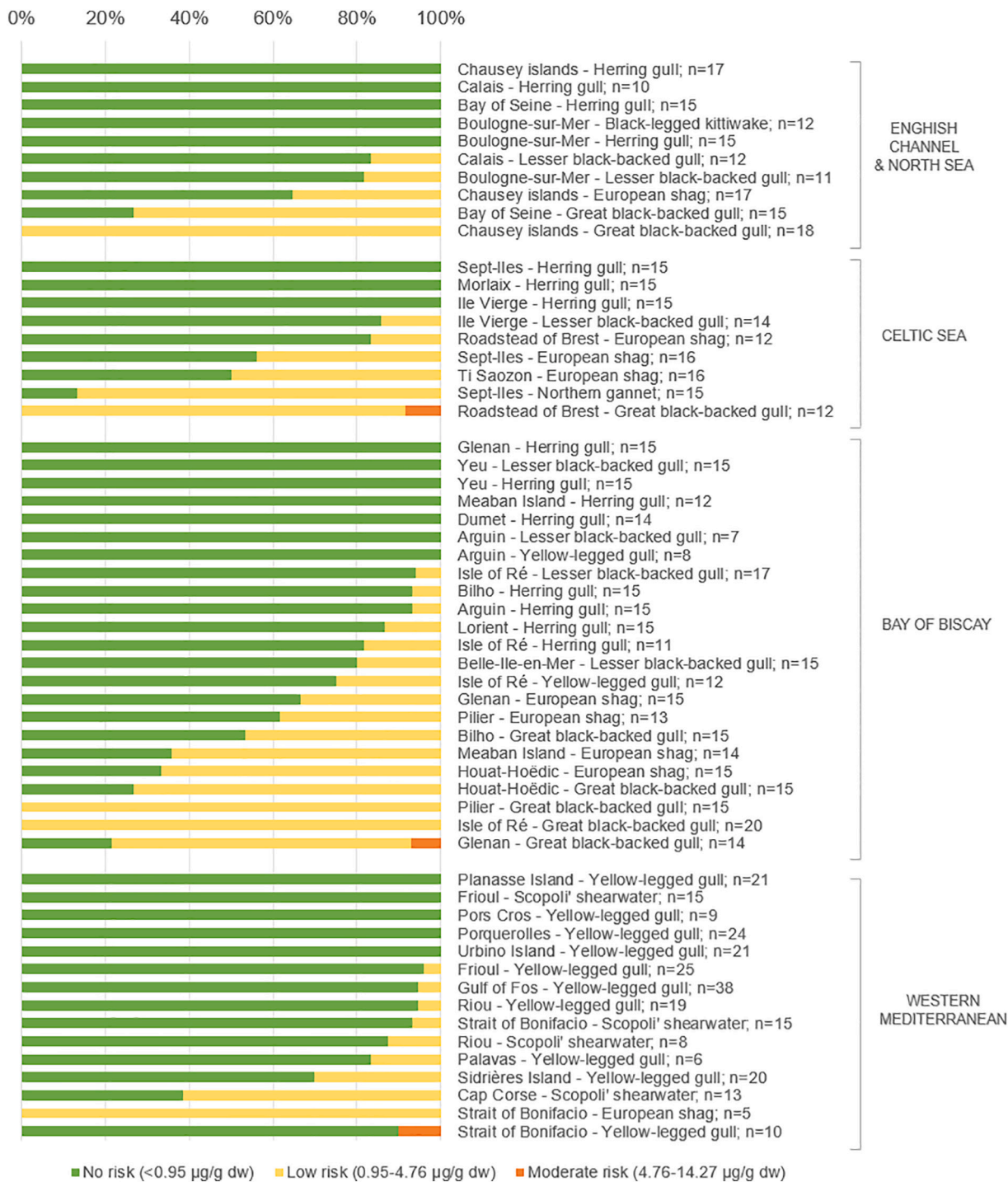


Fig. 5. Overview of the percentage of individual seabird chicks per site along the French coasts, that are at risk for Hg toxicity according to thresholds established for adults (Ackerman et al., 2016, converted to dry weight with moisture percentage of 79%, Eagles-Smith et al., 2008). Thresholds are classified into three categories: $<0.95\ \mu\text{g g}^{-1}\ \text{dw}$ (green; below known effect levels), $0.95\text{--}4.76\ \mu\text{g g}^{-1}\ \text{dw}$ (yellow; low risk) and $4.76\text{--}14.27\ \mu\text{g g}^{-1}\ \text{dw}$ (orange; moderate risk). Brackets indicate marine subregions.

risk (0.95–4.76 $\mu\text{g g}^{-1}$ dw, converted) and only three individuals (<0.5% of chicks) at moderate risk (4.76–14.27 $\mu\text{g g}^{-1}$ dw converted). However, chicks may differ from adults in their susceptibility to Hg, particularly during the growth phase, which corresponds to the development of the nervous system. Importantly, sublethal effects have been reported even at concentrations considered at no risk in chicks of lesser b-b gulls ($\sim 0.25 \mu\text{g g}^{-1}$ dw, Santos et al., 2020) and shearwaters (0.6 to 1.0 $\mu\text{g g}^{-1}$ dw, Costantini et al., 2020). Santos et al. (2020) showed changes in energy metabolism (decrease of total proteins, carbohydrates, and lipids in blood), as well as an increase in oxidative stress in chicks of lesser b-b gulls at $\sim 0.25 \mu\text{g g}^{-1}$ dw. In our study, 77% of individuals ($n = 651$) exceeded this level. Moreover, Hg concentrations (0.6 to 1.0 $\mu\text{g g}^{-1}$ dw) were also associated with high oxidative stress in chicks of shearwaters (Costantini et al., 2020). Exposure to Hg may raise ecotoxicological concerns, but Hg can be detoxified by selenium (Se), a trace element that plays a protective role against Hg toxicity (Ikemoto et al., 2004; Kim et al., 1996). Additional studies on Se could help evaluate whether species and geographical variability in this element could compensate Hg contamination (Cruz-Flores et al., 2024). Further studies are also now required to better understand the consequences of Hg contamination, particularly during the developmental stages, but also on lifetime fitness.

5. Conclusions

Our study provides novel information on Hg contamination in seabirds from metropolitan France and describes its main drivers for a large panel of seabird species with various trophic ecologies. Hg variation was explained mainly by extrinsic trophic drivers. Trophic position was a key contributor, with higher $\delta^{15}\text{N}$ values resulting in higher blood Hg concentrations. Chicks from species with diverse trophic ecologies appear to be good bioindicators of Hg transfer to predators in coastal ecosystems. We also provided a unique large-scale description of Hg burdens in seabird chicks, which points to geographically homogeneous variation in Hg contamination along the French coasts. Even if most chicks were classified to be at low risk according to adult thresholds, concentrations in excess of sublethal effects were found, calling for future studies on the effects of Hg on early life stages.

CRedit authorship contribution statement

Prescillia Lemesle: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Alice Carravieri:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Gauthier Poiriez:** Project administration, Investigation. **Romain Batard:** Investigation. **Aurélien Blanck:** Resources, Investigation. **Armel Deniau:** Investigation. **Gilles Faggio:** Investigation. **Jérôme Fort:** Writing – review & editing, Resources. **Fabrice Gallien:** Investigation. **William Jouanneau:** Writing – review & editing, Investigation. **Gilles le Guillou:** Investigation. **Carole Leray:** Investigation. **Karen D. McCoy:** Writing – review & editing, Resources, Funding acquisition. **Pascal Provost:** Investigation. **Marie-Catherine Santoni:** Investigation. **Manrico Sebastiano:** Writing – review & editing, Investigation. **Alain Ward:** Investigation. **Olivier Chastel:** Writing – review & editing, Supervision, Resources, Investigation. **Paco Bustamante:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.175857>.

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