



Mercury contamination in gull chicks raised in natural vs. urban habitats

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ABSTRACT

Anthropogenic activities have increased pressure on marine ecosystems through the continuous overflow of pollutants like mercury (Hg). Seabirds, particularly chicks, serve as effective local bioindicators of marine ecosystem health. This study assessed the influence of trophic ecology (inferred from $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values) and colony location on Hg concentrations in the blood of yellow-legged (YLG, *Larus michahellis*) and Audouin's (AG, *Ichthyophaga audouinii*) gull chicks raised in natural (YLG, AG) vs. urban habitats (YLG). We report the highest blood Hg concentrations ever documented in chicks of these species raised in natural habitats (range: AG, 0.59–4.19 $\mu\text{g g}^{-1}$ dw; YLG, 0.74–2.82 $\mu\text{g g}^{-1}$ dw), while urban-raised YLG chicks exhibited up to 2-fold lower Hg concentrations (range: YLG, 0.22–1.23 $\mu\text{g g}^{-1}$ dw). Interestingly, a positive association between trophic position (reflected by $\delta^{15}\text{N}$) and Hg concentrations was observed in urban YLG chicks but not in chicks raised in natural habitats. On the other hand, body mass was negatively associated to blood Hg concentrations in gull chicks raised in natural colonies. Overall, these results highlight the roles of the ecological context, trophic position, and body mass increase along the nestling period on driving Hg exposure in seabird chicks. We stress for further baseline contaminant studies and for more research on how these Hg concentrations could impact the physiology and development of chicks, despite the current Hg concentrations pose an apparent low risk.

Mercury (Hg) is a non-essential element widely present in the environment. Anthropogenic activities, including gold mining and fossil fuel combustion, have increased atmospheric Hg concentrations by 300–500% since late 19th century (Outridge et al., 2018). This significant rise has facilitated the global dispersion of Hg and its subsequent deposition into marine ecosystems (AMAP/UNEP, 2013; Fitzgerald et al., 2007). In marine environments, Hg is predominantly converted into methylmercury (MeHg) by microorganisms (Podar et al., 2015). MeHg is the most toxic organic form of Hg known to undergo bioaccumulation in biota (increase of concentrations along time) and to biomagnify up the food webs, with increasing concentrations in tissues of organisms at higher trophic levels (Chen et al., 2008; Seco et al., 2021). Thus, top predators like seabirds can be exposed to considerable concentrations of Hg via their diet (Carravieri et al., 2014a, 2014b;

Cherel et al., 2018).

Gulls are opportunistic seabirds used as bioindicators of coastal marine contamination (Distefano et al., 2022; Patier et al., 2024; Sebastiano et al., 2021). With increasing urbanisation and the corresponding rise in predictable anthropogenic food sources, gulls have benefited by expanding and establishing colonies in urban areas (Duhem et al., 2008; Pais de Faria et al., 2021a). This shift has allowed the additional use of gulls as biomonitors of urban and terrestrial contamination due to their exposure to novel contaminants, which can have potential physiological consequences at all life-stages (Nos et al., 2024; Veríssimo et al., 2024). Seabird chicks are commonly used as local bioindicators of contamination because they are fed with prey collected in the vicinity of the colonies (Binkowski et al., 2021; Carravieri et al., 2020). Foraging habitats and trophic ecology of seabirds are pivotal for

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ecotoxicological assessments, as contaminants are mainly derived from their diet. The combined analysis of stable isotopes of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ provides insights into foraging habitats (benthic vs. pelagic and marine vs. terrestrial) and trophic positions, respectively (Kelly, 2000; Newsome et al., 2007). Chicks with higher $\delta^{15}\text{N}$ values often exhibit elevated blood Hg concentrations, suggesting individuals feeding at higher trophic levels are more exposed to Hg (Binkowski et al., 2021; Lemesle et al., 2024a). In contrast, $\delta^{13}\text{C}$ values have not consistently explained variations in Hg concentrations of chicks across species (Binkowski et al., 2021; Costantini et al., 2020; Lemesle et al., 2024a).

In this study, we investigated Hg concentrations in the blood of gull chicks raised in two environments: natural vs. urban breeding colonies. We focused on chicks of two gull species, the Audouin's gull (AG, *Ichthyophaga audouinii*) and the yellow-legged gull (YLG, *Larus michahellis*), of approximately 2 weeks-old to assess the impact of the trophic position and of the feeding habitats on blood Hg concentrations. Given that the ecological context can influence gull diets (Calado et al., 2021; Pais de Faria et al., 2021b), we anticipated different $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between urban and natural environments due to different isotope baseline values (Elliott et al., 2021; Lavoie et al., 2013). We predicted 1) lower blood Hg contamination in urban YLG chicks due to reduced Hg biomagnification in terrestrial food webs (Lavoie et al., 2013); 2) similar Hg concentrations in AG and YLG chicks from natural colonies, as previous findings indicated comparable blood Hg concentrations in adults from the same colonies (dos Santos et al., 2024); and 3) higher $\delta^{15}\text{N}$ values would correlate with increasing Hg concentrations, while $\delta^{13}\text{C}$ values might not effectively explain Hg variations in chicks from both species (Binkowski et al., 2021; Lemesle et al., 2024a; Patier et al., 2024).

From May to June 2021–2023, we collected a blood sample (~ 400 μL) from 68 gull chicks raised in three breeding colonies: 20 AG and 19 YLG from Deserta Island, Algarve (36°57'N, 7°53'W), and 29 YLG from the city of Porto (41°08'N, 8°36'W). Chicks from Porto were monitored since their hatching day, while those sampled at Deserta were randomly caught. Thus, in Deserta body mass was used as proxy of chick age (Lequette and Weimerskirch, 1990; Nelson, 1964). However, we acknowledge that chick body mass can fluctuate according to meal delivery. AG chicks were estimated with 17 ± 2 days of age (range 15–21 days), YLG chicks from Deserta were estimated with 13 ± 2 days of age (range 9–18 days), and YLG chicks from Porto had 12 ± 3 days of age (range 8–19 days). Blood samples were centrifuged and red blood cells (hereafter 'blood') were freeze-dried for the quantification of Hg (further expressed in $\mu\text{g g}^{-1}$ dw) and for stable isotopes analysis of $\delta^{13}\text{C}$ ($^{13}\text{C}/^{12}\text{C}$, in ‰) and $\delta^{15}\text{N}$ ($^{15}\text{N}/^{14}\text{N}$, in ‰). Briefly, blood Hg was measured with an Altec AMA 254 spectrophotometer (aliquot mass: 0.68 ± 0.19 mg dw) with a limit of detection of 0.1 ng (Bustamante et al., 2006). Samples were run in duplicate-triplicate until reaching a relative standard deviation below 10%. Blanks were run at the beginning of each set of samples, while the certified reference material (CRM) – TORT-3 lobster hepatopancreas (Hg certified concentration: 0.292 ± 0.022 $\mu\text{g g}^{-1}$ dw) – was ran every 15 samples to check the accuracy of the method. CRM measured values were 0.296 ± 0.003 $\mu\text{g g}^{-1}$ dw ($N = 8$), corresponding to a recovery rate of $102.1 \pm 0.9\%$. Results are given as mean $\pm$ SD. Relative abundances of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were measured using a continuous flow mass spectrometer (Delta V Plus with a ConFlo IV interface, Thermo Scientific, Bremen, Germany) coupled to an elemental analyser (Thermo Scientific EA 1112) following dos Santos et al. (2024). Results are given using the delta (δ) notation, calculated using the equation: $\delta X = [(R_{\text{Sample}} / R_{\text{Standard}}) - 1] \times 1000$, where X stands by ^{13}C or ^{15}N , R corresponds to $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ ratio, and standard values correspond to Vienna PeeDee Belemnite for carbon, and atmospheric N_2 for nitrogen. Analytical precision (standard deviation associated with replicate runs of USGS-61 and USGS-62) was <0.10 ‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

Data exploration, visualisation, and statistical computations were carried out in R 4.3.1 (R Core Team, 2023). Before comparing blood $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, as well as Hg concentrations between colonies and

years, each variable was tested for normality and homoscedasticity, using Shapiro-Wilk and Bartlett tests, respectively. Each unique combination of colony (AG, YLG from Deserta, YLG from Porto) and sampling year (2021, 2022, 2023) was treated as a distinct group to avoid confounding effects that could arise from differences in sampling years across colonies, resulting in a total of six groups: "YLG Porto 2021", "YLG Porto 2022", "YLG Deserta 2022", "YLG Deserta 2023", "AG Deserta 2022", and "AG Deserta 2023". Each of these combinations was analysed as a separate group to control for potential temporal variation in factors such as diet, environmental conditions, or exposure to contaminants (like Hg). By following this data structure, we aimed to ensure that any differences observed among colonies were not confounded by the year in which samples were collected. However, we acknowledge that splitting groups by year may reduce sample sizes within groups and potentially affect the statistical power of inter-group comparisons (see Table 1 for sample sizes). One-way ANOVA, or Kruskal-Wallis (K-W) for non-parametric data distribution, was used to assess differences on blood $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, as well as on Hg concentrations, among groups. Whenever differences were detected, post-hoc Tukey tests or pairwise tests were applied on significant results (significance level of $\alpha < 0.05$), with p -value adjustments using Bonferroni correction. In addition, we used Stable Isotope Bayesian Ellipses in R (SIBER package, Jackson et al., 2011) to compute isotopic niches separately for each group. Bayesian standard ellipse and its areas (SEA_B) were used to draw group-specific ellipses ($\delta^{13}\text{C} - \delta^{15}\text{N}$) and to test for differences on niche width, whereas SEA corrected for small sample sizes (SEA_C) were computed for visualisation.

To determine whether blood $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values – indicative of

Table 1

Blood Hg concentrations ($\mu\text{g g}^{-1}$ dw), carbon ($\delta^{13}\text{C}$), nitrogen ($\delta^{15}\text{N}$) isotope values (‰), and body mass (g) of gulls' chicks sampled from 2021 to 2023: sample size (N), mean $\pm$ SD, and range values (minimum/maximum) are expressed for each sampling year and for each sampling colony. Different letters in superscript indicate statistical differences ($\alpha < 0.05$) on Hg, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$.

	Hg ($\mu\text{g g}^{-1}$ dw)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Body mass (g)
Deserta Island				
Audouin's gull (N = 20)				
2022 (N = 10)	1.77 $\pm$ 1.03 ^b (0.59/ 4.19)	−19.3 $\pm$ 0.2 ^a (−19.5/ −19.0)	12.6 $\pm$ 1.6 ^b (9.4/14.1)	259 $\pm$ 83 (150/ 400)
2023 (N = 10)	2.07 $\pm$ 0.55 ^b (1.05/ 2.85)	−19.0 $\pm$ 0.2 ^b (−19.3/ −18.8)	14.2 $\pm$ 0.2 ^c (13.8/ 14.6)	258 $\pm$ 34 (215/ 325)
Yellow-legged gull (N = 19)				
2022 (N = 10)	1.49 $\pm$ 0.46 ^b (0.75/ 2.39)	−18.6 $\pm$ 0.4 ^b (−19.3/ −17.8)	12.2 $\pm$ 0.3 ^b (11.6/ 12.5)	367 $\pm$ 140 (180/ 580)
2023 (N = 9)	2.29 $\pm$ 0.50 ^b (1.41/ 2.83)	−18.7 $\pm$ 0.4 ^b (−19.0/ −17.8)	12.3 $\pm$ 0.3 ^b (11.8/ 12.5)	313 $\pm$ 91 (170/ 480)
Porto				
Yellow-legged gull (N = 29)				
2021 (N = 13)	0.45 $\pm$ 0.15 ^a (0.26/ 0.82)	−19.6 $\pm$ 0.8 ^a (−21.6/ −18.7)	11.4 $\pm$ 0.7 ^{ab} (10.1/ 12.8)	248 $\pm$ 54 (175/ 370)
2022 (N = 16)	0.55 $\pm$ 0.27 ^a (0.22/ 1.23)	−19.6 $\pm$ 0.7 ^a (−21.1/ −18.9)	10.9 $\pm$ 0.6 ^a (10.1/ 11.8)	340 $\pm$ 126 (130/ 585)

feeding habitat and trophic position, respectively (Newsome et al., 2007) – affected Hg concentrations on chicks, we employed regression-type models (generalised linear models, GLM, or mixed effect models, GLMM) adapted to data distribution using *lme4* R package (Bates et al., 2015). Previous research revealed that YLGs breeding in Porto occupy a distinct isotopic niche compared to YLG and AG adults and chicks from Deserta (dos Santos et al., 2024; Pais de Faria et al., 2021b). Thus, we divided the analysis into two separate models:

1) a GLMM using only the YLG chicks from Porto, using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, and its interaction ' $\delta^{13}\text{C} \times \delta^{15}\text{N}$ ', as predictors and Hg concentrations as the response variable. Chick age was included as a predictor since it may impact chick blood Hg concentrations (Ackerman et al., 2011; Wenzel et al., 1996), regardless of their diet (Santos et al., 2020). The year was not included in this model because there were no annual differences in any of the predictors neither on blood Hg concentrations in these chicks. Also, as we sampled chicks from the same nest, this was added as a random factor to avoid pseudo replication issues (Zuur et al., 2009). The interaction terms ' $\delta^{15}\text{N} \times \text{Age}$ ' and ' $\delta^{13}\text{C} \times \text{Age}$ ' were also added since there is evidence of a gradual shift on chicks' diet along their growth in Porto (Pais de Faria et al., 2021b). Full model: $\text{Hg} \sim \delta^{13}\text{C} \times \delta^{15}\text{N} + \delta^{13}\text{C} \times \text{Age} + \delta^{15}\text{N} \times \text{Age} + (1 | \text{Nest})$.

2) a GLM using YLG and AG chicks from Deserta, using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, but with the species as an additional predictor, to account for species-specific variations, and chicks' body mass to control for its known impact on Hg changes along chick growth (Ackerman et al., 2011; Lemesle et al., 2024b), ensuring independent estimation of the effects of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and species. Year was included as a predictor variable since there were annual variations on blood $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of AG. Interaction terms ' $\delta^{13}\text{C} \times \text{Year}$ ', ' $\delta^{15}\text{N} \times \text{Year}$ ', ' $\text{Year} \times \text{Species}$ ', ' $\delta^{13}\text{C} \times \text{Species}$ ', and ' $\delta^{15}\text{N} \times \text{Species}$ ' were also included. However, ' $\delta^{13}\text{C}:\delta^{15}\text{N}$ ' was not included in the model due to known differences between AG's and YLG's diet (Calado et al., 2021), which could bias model predictions. Full model: $\text{Hg} \sim \delta^{13}\text{C} \times \text{Year} + \delta^{15}\text{N} \times \text{Year} + \text{Year} \times \text{Species} + \delta^{13}\text{C} \times \text{Species} + \delta^{15}\text{N} \times \text{Species} + \text{Body Mass}$.

Model selection was carried out through manual backward elimination, starting with the full model and sequentially removing non-significant interactions or single variables. The candidate models were ranked according to the Akaike's Information Criterion corrected for small sample size (AICc) (Burnham and Anderson, 2002). The model with the lowest AICc value, i.e., ΔAICc equal to 0, was considered the best-fit model and retained for further interpretation (Burnham and Anderson, 2002). Residuals were checked for normality, homoscedasticity, and outliers using the *performance* (Lüdtke et al., 2021) and *DHARMa* R packages (Hartig, 2022), prior to model selection. Predicted values and confidence intervals (CI) derived from the best models were extracted using the 'ggpredict' function from *ggeffects* R package (Lüdtke, 2018) and visualised using *ggplot2* R package (Wickham et al., 2023).

The mean $\pm$ SD values for Hg, stable isotopes, and body mass are presented in Table 1. Very strong differences in Hg concentrations, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ values were observed among colonies and years (K-W, $34.97 < \chi^2 < 50.08$, $p < 0.001$; Table 1). Specifically, $\delta^{13}\text{C}$ values were higher in YLG chicks from Deserta Island compared to urban YLG and AG chicks sampled in 2022 ($0.001 < p < 0.03$; Fig. 1A, Table 1). $\delta^{15}\text{N}$ values were higher in AG chicks sampled in 2023 than in all other groups ($p < 0.001$), including AG chicks sampled in 2022 ($p = 0.03$). Urban YLG chicks in 2022 had lower $\delta^{15}\text{N}$ values than YLG chicks from Deserta Island ($p < 0.001$; Fig. 1B, Table 1). Blood Hg concentrations were lower in urban YLG chicks compared to YLG and AG chicks from Deserta Island ($p < 0.001$; Fig. 1C, Table 1). Bayesian estimation of standard ellipse area (SEA_B) indicated that urban YLG chicks exhibited the broadest isotopic niches, regardless of the year (Fig. S1). Remarkably, AG chicks sampled in 2022 showed a similar isotopic niche width to urban YLG chicks, a pattern not observed in AG chicks sampled in 2023 (Fig. S1).

Notably, we found the highest blood Hg concentrations ever reported for AG and YLG chicks raised in natural colonies. Blood Hg

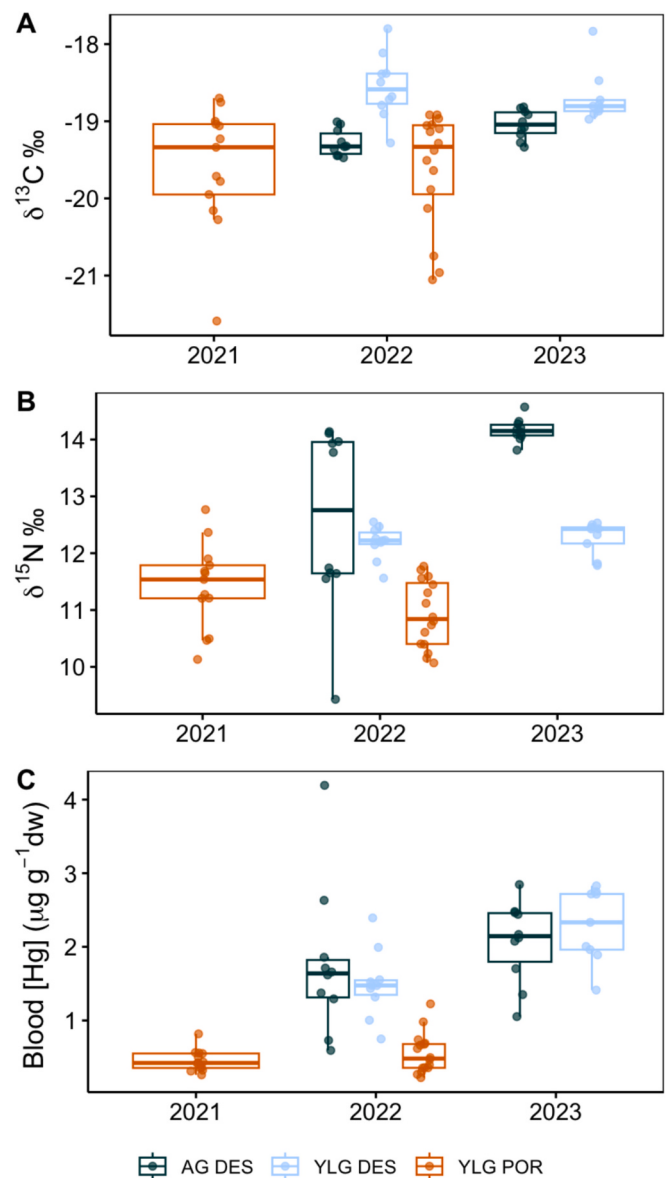


Fig. 1. Jitter plots of $\delta^{13}\text{C}$ (A) and $\delta^{15}\text{N}$ (B) values (‰), as well as Hg concentrations (C, $\mu\text{g g}^{-1} \text{ dw}$), in the blood of gull chicks sampled in three years and reared in three colonies (colours) located in the western and southern coasts of Portugal. Values are median, 25th and 75th percentiles, range (straight solid lines), and the real data points. Abbreviations: AG, Audouin's gull; YLG: yellow-legged gull; DES, Deserta Island (natural colony); POR: Porto (urban colony). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

concentrations in AG chicks from Deserta Island were 3-fold higher than those recorded in AG chicks from Southeast Spain (mean $\pm$ SD: $0.61 \pm 0.14 \mu\text{g g}^{-1} \text{ dw}$; Espín et al., 2016). Similarly, YLG chicks from Deserta Island exhibited blood Hg concentrations 2 to 9-fold higher than those recorded in YLG chicks from colonies along the North and West coasts of France and the Mediterranean (mean range: $0.22\text{--}1.69 \mu\text{g g}^{-1} \text{ dw}$; Jouanneau et al., 2022; Lemesle et al., 2024a; Patier et al., 2024). In contrast, urban YLG chicks showed low and comparable blood Hg concentrations to those of the aforementioned colonies, as well as to herring (*L. argentatus*) and lesser black-backed gulls (*L. fuscus*) from the French Atlantic and Channel coasts (mean range: $0.11\text{--}0.77 \mu\text{g g}^{-1} \text{ dw}$; Binkowski et al., 2021; Jouanneau et al., 2022; Lemesle et al., 2024a; Santos et al., 2020), and to urban-nesting black-legged kittiwakes (*Rissa tridactyla*) from the Channel coast (mean $\pm$ SD: $0.43 \pm 0.07 \mu\text{g g}^{-1} \text{ dw}$;

Lemesle et al., 2024b). Nevertheless, we acknowledge these studies have sampled well-feathered chicks, which tend to exhibit lower Hg concentrations compared to younger chicks (discussed below).

In Porto, YLG chicks are fed with both terrestrial and marine resources, with adults adjusting the marine/terrestrial prey ratio delivered to chicks during the rearing period (Pais de Faria et al., 2021b). Younger chicks (up to 20 days old) receive 60–80% marine prey, while older chicks consume up to 65% anthropogenic resources (Pais de Faria et al., 2021b). Even at younger stages, urban YLG chicks consume 20–40% of anthropogenic resources (Pais de Faria et al., 2021b), possibly explaining why their blood Hg concentrations are lower than chicks from Deserta Island. At Deserta, AG and YLG adults extensively exploit marine prey, including pelagic fish and mesopelagic/demersal fish discarded from fishing vessels (Calado et al., 2021; Matos et al., 2018). Mesopelagic and demersal fish are often more Hg-enriched than pelagic species (Chouvelon et al., 2012; Choy et al., 2009) due to higher rates of inorganic Hg methylation into MeHg in low-oxygen waters mediated by

microbial activity (Blum et al., 2013). The large differences in Hg concentrations between urban and natural colonies may be attributed to the greater bioavailability of MeHg in marine ecosystems (Blum et al., 2013; Eagles-Smith et al., 2016), and the general longer and more complex food webs in marine environments compared to terrestrial or freshwater systems (Lavoie et al., 2013; Post, 2002), which promote higher Hg biomagnification rates (Eagles-Smith et al., 2016).

In seabird chicks, higher Hg concentrations were associated with higher $\delta^{15}\text{N}$ values, which is closely related to the consumption of higher trophic level marine prey (e.g., Binkowski et al., 2021; Blévin et al., 2013). In the present study, chick blood $\delta^{15}\text{N}$ values were within the upper range of adults during the incubation period (dos Santos unpublished data), indicating that chicks primarily received prey of higher trophic levels. This dietary shift often occurs after hatching, reflecting a change in energy requirements, a common phenomenon observed in seabirds, particularly in gulls (Annett and Pierotti, 1989; Pais de Faria et al., 2021b; Pierotti and Annett, 1987). In urban YLG chicks, $\delta^{15}\text{N}$ was

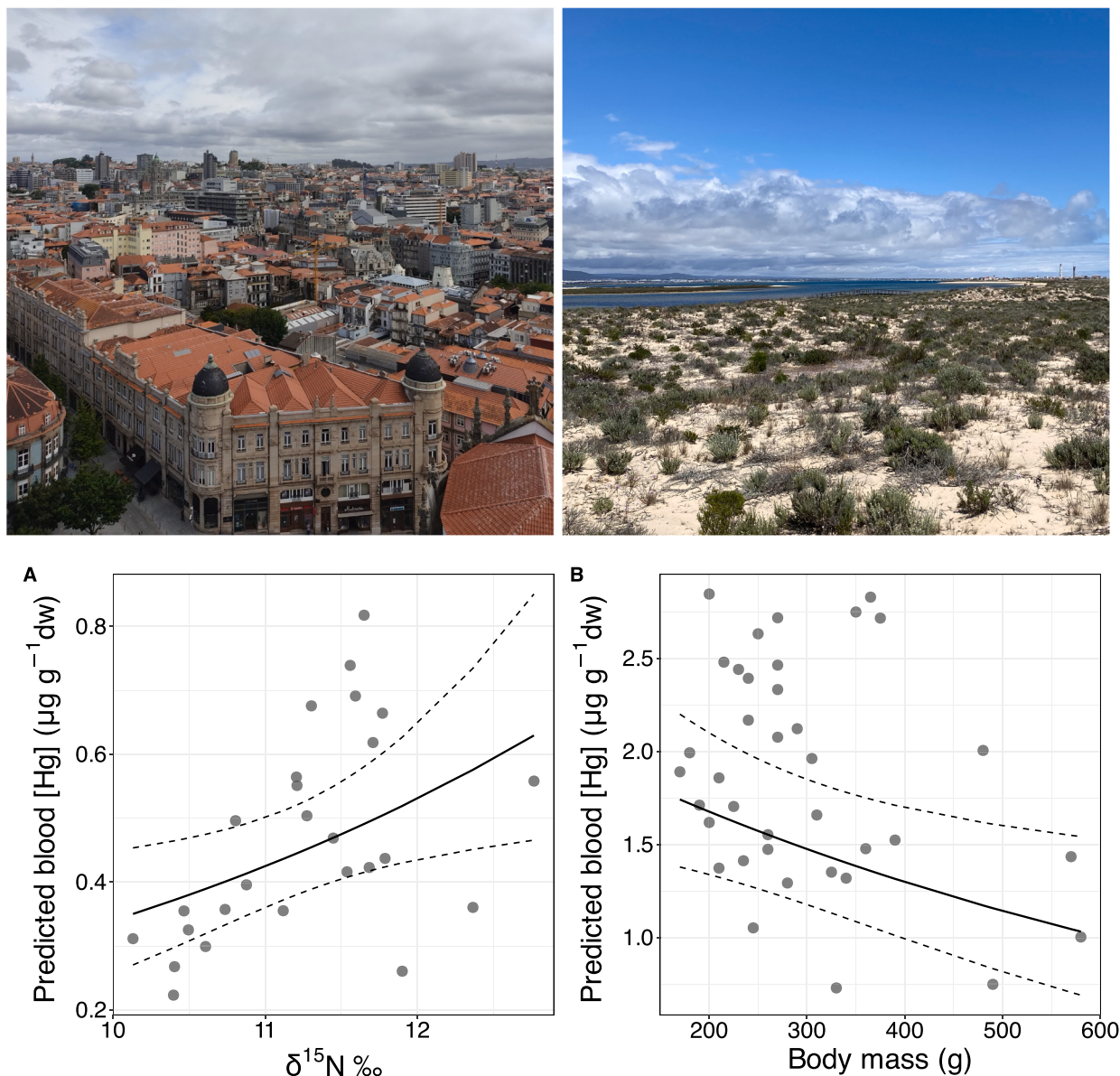


Fig. 2. Predicted blood Hg concentrations ($\mu\text{g g}^{-1}\text{dw}$) in relation to: (A) $\delta^{15}\text{N}$ values in urban YLG chicks and (B) body mass in AG and YLG chicks from Deserta Island. A) Hg increased with increasing $\delta^{15}\text{N}$ values; B) Hg decreased with increasing body mass, a proxy of chick age. Solid lines represent the predicted lines for the significant relationship; solid points represent the predicted Hg concentrations; dashed lines represent the 95% confidence intervals, CI. Photographs represent the urban breeding colony (Porto, on the left) and the natural breeding colony (Deserta Island, on the right).

the strongest positive predictor of blood Hg concentrations (GLMM, $\beta \pm \text{SE}$, 0.24 ± 0.09 , $t = 2.65$, $p = 0.008$, $\text{AICc} = -26.28$; Fig. 2A, Table 2). Conversely, the body mass of Deserta Island chicks was negatively associated with Hg concentrations (GLM, -0.001 ± 0.001 , $t = -2.58$, $p = 0.02$, $\text{AICc} = 64.91$; Fig. 2B, Table 2). Although Hg biomagnification rates are generally lower in terrestrial systems, they can vary depending on local ecological dynamics (Eagles-Smith et al., 2016). Urban YLG chicks with higher trophic positions (with higher $\delta^{15}\text{N}$ values), had higher blood Hg concentrations, consistent with findings from other gull populations (Binkowski et al., 2021; Jouanneau et al., 2022; Patier et al., 2024). This shows that, even in urban environments with low Hg concentrations, biomagnification can still occur. Our results contrast with a recent study on urban black-legged kittiwake chicks, where no

Table 2

Summary outputs including model specification, sample size (n), and Akaike's Information Criteria (AIC) are presented for the best three ranked models and for the null model. The best ranked model outputs are specified. Significant effects are shown in bold, and the number of asterisks indicate the increasing level of significance ($\alpha < 0.05$, $\alpha < 0.01$, $\alpha < 0.001$). Abbreviations: k , number of parameters; AICc, Akaike's information criterion corrected for small sample sizes; ΔAICc , difference between AICc of the specific model and the best ranked model; w_i , AICc weights; Exp. Dev., explained deviance; BM, body mass; Sp, species; Y, year.

Model specifications	k	AICc	ΔAICc	w_i	Exp. Dev.
Porto ($n = 26$)^a - GLMM Gamma family ('log' link)					
Full model: Blood Hg $\sim \delta^{13}\text{C} \times \delta^{15}\text{N} + \delta^{15}\text{N} \times \text{Age} + \delta^{13}\text{C} \times \text{Age} + (1 \mid \text{Nest})$					
$\delta^{15}\text{N} + \text{Age} + (1 \mid \text{Nest})$	4	-26.98	0.00	0.75	0.89
$\delta^{15}\text{N} + \delta^{13}\text{C} + \text{Age} + (1 \mid \text{Nest})$	5	-23.58	3.40	0.14	0.89
$\delta^{15}\text{N} + \delta^{13}\text{C} \times \text{Age} + (1 \mid \text{Nest})$	6	-22.65	4.34	0.09	0.91
Null model	2	-19.80	7.19	0.02	0.00
	$\beta \pm \text{SE}$	z value	$p(> z)$		
Intercept	-2.92 $\pm$ 1.07	-2.73	0.006	**	
$\delta^{15}\text{N}$	0.22 $\pm$ 0.09	2.48	0.013	*	
Age	-0.03 $\pm$ 0.02	-1.37	0.170		
Deserta ($n = 37$)^b - GLM Gamma family ('log' link)					
Full model: Blood THg $\sim \delta^{13}\text{C} \times \text{Sp} + \delta^{15}\text{N} \times \text{Sp} + \delta^{15}\text{N} \times \text{Y} + \delta^{13}\text{C} \times \text{Y} + \text{Y} \times \text{Sp} + \text{BM}$					
$\delta^{13}\text{C} + \delta^{15}\text{N} \times \text{Y} + \text{Y} \times \text{Sp} + \text{BM}$	8	64.91	0.00	0.79	0.50
$\delta^{15}\text{N} \times \text{Y} + \delta^{13}\text{C} \times \text{Sp} + \text{Y} \times \text{Sp} + \text{BM}$	9	68.27	3.35	0.15	0.51
Null model	2	70.86	5.95	0.04	0.00
$\delta^{13}\text{C} \times \text{Sp} + \delta^{15}\text{N} \times \text{Sp} + \delta^{15}\text{N} \times \text{Y} + \text{Y} \times \text{Sp} + \text{BM}$	10	71.94	7.02	0.02	0.52
	$\beta \pm \text{SE}$	t value	$p(> t)$		
Intercept	2.43 $\pm$ 3.11	0.78	0.44		
$\delta^{15}\text{N}$	-0.11 $\pm$ 0.08	-1.48	0.15		
$\delta^{13}\text{C}$	0.01 $\pm$ 0.15	0.07	0.94		
Body Mass	-0.001 $\pm$ 0.001	-2.58	0.02	*	
Year(2023)	7.41 $\pm$ 3.75	1.97	0.06		
Species(YLG)	-0.007 $\pm$ 0.184	-0.04	0.97		
Year(2023)xSpecies(YLG)	-0.94 $\pm$ 0.52	-1.82	0.08		
$\delta^{15}\text{N} \times \text{Year}(2023)$	-0.49 $\pm$ 0.27	-1.85	0.07		

^a One nest with three chicks was removed from the dataset due to outliers in Hg concentrations.

^b Two AG chicks were removed from the dataset due to outliers in $\delta^{15}\text{N}$ values and Hg concentrations.

relationship between blood Hg and $\delta^{15}\text{N}$ values was observed (Lemesle et al., 2024b). However, a negative association between Hg and body mass (a proxy of chick age) was observed in kittiwakes, similarly to our gulls from Deserta Island. In our study, the effect of chick growth may have weakened the relationship between $\delta^{15}\text{N}$ values and Hg concentrations in chicks from Deserta Island. Previous studies have shown that blood Hg concentrations decrease significantly as chicks grow, due to depuration of Hg into growing feathers and dilution as body size increases (Ackerman et al., 2011; Santos et al., 2020; Wenzel et al., 1996). However, this effect was possibly diluted by the stronger effect of $\delta^{15}\text{N}$ values in explaining Hg variations in urban YLG chicks.

Several factors may explain this distinct pattern: 1) the narrow range of blood $\delta^{13}\text{C}$ values in gulls from Deserta Island (AG: -19.5 to -18.8 ‰; YLG: -19.3 to -17.8 ‰) suggests that chicks received food from similar foraging habitats, indicating no major shift in feeding areas or available resources, despite significant differences for AG; 2) the narrow range of blood $\delta^{15}\text{N}$ values of YLG (both years) and AG (in 2023) suggests that chicks were fed similar trophic level prey, potentially restricting the isotopic niche. This could have limited the ability to detect associations between Hg and $\delta^{15}\text{N}$, as seen in YLG chicks from Porto. 3) In 2022, the broader $\delta^{15}\text{N}$ range in AG chicks (Fig. S1) indicates variations in the trophic position, with some chicks consuming lower trophic level prey (e.g., marine invertebrates), while others were fed higher trophic level prey (e.g., fish; Ceia et al., 2014, 2023; Moreno et al., 2010). Indeed, AG adults increased the variety of prey on their diet in 2022 compared to the previous years, feeding more on Henslow's swimming crab and insects and less on demersal fish (Pereira et al., 2022). While prey consumed by parents may not necessarily reflect that delivered to their offsprings, this may help explain the broader isotopic niche of AG chicks in 2022 (especially due to the enlargement of $\delta^{15}\text{N}$ range). Also, this suggests parental differences in foraging behaviour, with some specialising in higher trophic level prey and others being more opportunistic or constrained by factors such as prey availability or competition for fishery discards (Calado et al., 2021; Moreno et al., 2011; Ramírez et al., 2021). This was supported by the broader range of Hg concentrations observed in AG chicks in 2022 (Hg range: 0.59 – 4.19 $\mu\text{g g}^{-1}$ dw) compared to those sampled in 2023 (Hg range: 1.05 – 2.84 $\mu\text{g g}^{-1}$ dw). These factors likely acted as confounding variables when evaluating the relationship between isotopic tracers and blood Hg concentrations in chicks from Deserta Island. As our chicks were on average 2 weeks old, we could expect a negligible impact of maternally transferred Hg on chick blood Hg concentrations. However, some influence from maternal transfer and growth rate cannot be entirely excluded, as both may affect the extent of Hg dilution in chick tissues. Therefore, future research should focus on the Hg that is effectively transferred from mothers to their chicks in these populations – for instance, using chick down Hg concentrations as a proxy of embryo's Hg burden (Ackerman and Eagles-Smith, 2009) or by collected a small blood amount at day 1 establishing the baseline for chick's blood Hg – and track Hg depuration along chick development (Ackerman et al., 2011; Wenzel et al., 1996).

Mercury is one of the most reputable contaminants with reported hazardous impacts on the physiology, endocrine and immune systems, behaviour, and reproduction of marine birds (reviewed by Chastel et al., 2022). In this study, we found some of the highest blood Hg concentrations reported for AG and YLG chicks worldwide. Chicks from Deserta Island exhibited blood Hg concentrations comparable to those of great black-backed gull (*L. marinus*) chicks from the French Atlantic and Channel coasts (mean range: 1.13 – 3.10 $\mu\text{g g}^{-1}$ dw; Binkowski et al., 2021; Jouanneau et al., 2022; Lemesle et al., 2024a). Based on toxicity benchmarks defined for adult birds (see Ackerman et al., 2016) – converted from 0.2 $\mu\text{g g}^{-1}$ wet weight, ww, considering a moisture percentage of 79% for blood (Eagles-Smith et al., 2008) – 44% of chicks were classified as at no risk category (< 0.95 $\mu\text{g g}^{-1}$ dw), and 56% fell into the low-risk category (0.95 – 4.76 $\mu\text{g g}^{-1}$ dw). Notably, 95% of those classified as at low risk were from Deserta Island, underlining the increased vulnerability of gulls reliant on marine prey, consistent with

findings for chicks along the French Atlantic and Mediterranean coastlines (Binkowski et al., 2021; Jouanneau et al., 2022; Lemesle et al., 2024a; Patier et al., 2024). Blood Hg concentrations between 1.2 and 2.0 $\mu\text{g g}^{-1}$ dw are known to impair physiological condition, endocrine function, and parental behaviour in adult seabirds (Ackerman et al., 2016; Chastel et al., 2022; Tartu et al., 2013, 2015, 2016), raising concerns about potential effects on chicks. Lemesle et al. (2024a) reinforced that these toxicity benchmarks were established for adults and that previous research have been overlooking the heightened susceptibility of chicks to Hg. Indeed, sublethal effects were reported in lesser black-backed gull chicks with blood Hg concentrations around 0.25 $\mu\text{g g}^{-1}$ dw (Santos et al., 2020), typically considered of no risk for adult seabirds (Ackerman et al., 2016, 2024; Chastel et al., 2022). Remarkably, in our study, 67 out of 68 chicks have exceeded this concentration. According to Santos et al. (2020), chicks with blood Hg around 0.25 $\mu\text{g g}^{-1}$ dw and fed with a marine diet exhibited increased oxidative stress and anaerobic metabolism, as well as decreased levels of energy metabolism (proteins, carbohydrates, and lipids). In another study, there was a positive association between blood Hg concentrations and oxidative stress in Scopoli's shearwater (*Calonectris diomedea*) chicks (mean range: 0.56–1.03 $\mu\text{g g}^{-1}$ dw) (Costantini et al., 2020). Therefore, we stress that blood Hg concentrations observed in our study raise significant ecotoxicological concerns. Further research using selenium – an essential element known to mitigate Hg toxicity (Cuvín-Aralar and Furness, 1991; Nigro and Leonzio, 1996) – could be crucial for determining whether chicks are particularly susceptible to the effects of Hg (Cruz-Flores et al., 2024; dos Santos et al., 2024), especially during their growth period.

In conclusion, our findings reveal higher Hg exposure in chicks from natural habitats compared to urban ones, consistent with previous reports for adults (dos Santos et al., 2024). Remarkably, the blood Hg concentrations observed rank among the highest reported for gull chicks globally, highlighting the need for site-specific risk assessments and tracking of potential Hg sources within the adults' foraging range. Additionally, as gulls increasingly nest in urban areas, their proximity to human settlements and waste sources exposes them not only to a wide range of contaminants, including organic pollutants, but also to pathogens such as antibiotic-resistant bacteria (Martín-Vélez et al., 2024a, 2024b). This raises public health concerns beyond ecotoxicological risks, as gulls could transport these pathogens away from urban centres, increasing the risk of zoonotic disease transmission between wildlife and humans.

CRediT authorship contribution statement

Ivo dos Santos: Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization, Writing – original draft. **Paco Bustamante:** Supervision, Resources, Funding acquisition, Conceptualization, Writing – original draft. **Maud Brault-Favrou:** Methodology, Writing – review & editing. **Adriana Domingues:** Investigation, Writing – review & editing. **Catarina Cascão:** Investigation, Writing – review & editing. **Joana Pais de Faria:** Investigation, Writing – review & editing. **Jorge M. Pereira:** Investigation, Writing – review & editing. **Nathalie Almeida:** Investigation, Writing – review & editing. **Ricardo Fernandes:** Investigation, Writing – review & editing. **Sara N. Veríssimo:** Investigation, Writing – review & editing. **Vitor H. Paiva:** Supervision, Resources, Funding acquisition, Conceptualization, Writing – original draft. **Jaime A. Ramos:** Supervision, Resources, Funding acquisition, Conceptualization, Writing – original draft.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2025.118317>.

Data availability

Data will be made available on request.

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