



## Mercury and other trace elements in gull chicks from a highly urbanised environment: effects on chick growth, physiology, and health condition

Ivo dos Santos <sup>a,b,\*</sup> , Vitor H. Paiva <sup>c</sup> , Ana C. Norte <sup>a</sup> , Carine Churlaud <sup>b</sup> , Maud Brault-Favrou <sup>b</sup> , Joana Pais de Faria <sup>a</sup> , Nathalie Almeida <sup>a</sup> , Ricardo Fernandes <sup>a</sup> , Jaime A. Ramos <sup>a</sup> , Paco Bustamante <sup>b,d</sup>

<sup>a</sup> University of Coimbra, MARE – Marine and Environmental Sciences Centre / ARNET – Aquatic Research Network, Department of Life Sciences, Calçada Martim de Freitas, 3000-456, Coimbra, Portugal

<sup>b</sup> Littoral Environnement et Sociétés (LIENSs), UMR 7266 CNRS - La Rochelle Université, 17000, La Rochelle, France

<sup>c</sup> University of Coimbra, CFE - Centre for Functional Ecology - Science for People & the Planet, Associate Laboratory TERRA, Department of Life Sciences, Calçada Martim de Freitas, 3000-456, Coimbra, Portugal

<sup>d</sup> Institut Universitaire de France (IUF), 1 rue Descartes, 75005, Paris, France



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### ABSTRACT

Coastal urbanisation has increased the exposure of urban-dwelling organisms to contaminants, such as trace elements (TEs) in opportunistic seabirds. This exposure may cause detrimental health effects, especially during sensitive early life-stages, before detoxification mechanisms are fully developed. We monitored yellow-legged gull (*Larus michahellis*) chicks in the city of Porto, Portugal, and collected down feathers and blood for mercury (Hg) and other TE analyses. We aimed to i) evaluate the effects of maternal exposure to Hg (inferred from down feathers) on early chick development, and ii) examine the impact of blood TE contamination on chick physiology and health condition. Aligned with previous research, blood Hg decreased as chicks aged (up to the 5<sup>th</sup> week), due to the growth dilution effect and depuration into the growing feathers. Additionally, down feather Hg concentrations showed a limited transfer of Hg from females to the embryo and was unrelated to hatching mass or early growth rate. While low maternal Hg exposure resulted in no detectable effects on early-life development or chick survival, dietary exposure (inferred from blood) to other TEs incurred physiological costs. Blood arsenic (As) and lead (Pb) concentrations were associated with a faster erythrocyte sedimentation rate, suggesting impaired immune response and inflammation. This highlights a dichotomy between trans-generational Hg transfer and post-hatching dietary exposure to Hg and other TEs. Future work should aim to define toxicity thresholds for TEs in seabird chicks, investigating the implications in the health since early life-stages, to better integrate the observed physiological responses.

### 1. Introduction

Trace elements (TEs) are chemical elements found in trace amounts in living organisms (<100 µg g<sup>-1</sup>; McNaught and Wilkinson, 1997). However, the rise of anthropogenic activities and the on-growing urbanisation have increased TEs emission and disposal into the global environment (Bai et al., 2023; Richir and Gobert, 2016; Vareda et al., 2019). While some of these elements are essential for biological processes – e.g., copper (Cu), iron (Fe), selenium (Se), and zinc (Zn) – others are non-essential, like cadmium (Cd), mercury (Hg), and lead (Pb), with

no known biological role and potentially toxic at very low concentrations (Kalisinska, 2019; Vallverdú-Coll et al., 2015). In organisms, a major concern regarding TEs is their strong tendency to lifetime bio-accumulation in tissues. Among them, Hg is known to also increase concentrations throughout trophic levels in a process called biomagnification (Lavoie et al., 2013). This process occurs mainly through its toxic organic form, methyl-Hg (MeHg), which is efficiently transferred along the food chain and retained in organisms. Consequently, top predators such as seabirds often exhibit elevated – and potentially hazardous – MeHg concentrations compared with their prey (Le Croizier

\* Corresponding author. University of Coimbra, MARE – Marine and Environmental Sciences Centre / ARNET – Aquatic Research Network, Department of Life Sciences, Calçada Martim de Freitas, 3000-456, Coimbra, Portugal, ORCID: 0000-0001-8415-1700 (Ivo dos Santos).

E-mail address: [ivo.d.santos@student.uc.pt](mailto:ivo.d.santos@student.uc.pt) (I. dos Santos).

et al., 2022; Seco et al., 2021).

Seabirds and other marine predators are mainly exposed to TEs through the ingestion of contaminated prey (Blévin et al., 2013; Carravieri et al., 2014b; Øverjordet et al., 2015). The use of seabirds as bio-indicators of chemical contamination of the marine environment has been recognised by the research community (Burger and Gochfeld, 2001; Furness and Camphuysen, 1997; Lopes et al., 2022). Over the past few decades, urbanisation of coastal areas has increased dramatically, forcing seabirds and other coastal species to leave or to adapt, with several species now inhabiting, foraging, and even breeding in urban environments. Gulls are among the seabird species best adapted to these novel habitats, taking advantage of human activity patterns (Méndez et al., 2020; Spelt et al., 2019, 2020) to exploit predictable anthropogenic food sources such as fishery discards, landfills, and leftovers from urban bins (Méndez et al., 2020; Pais de Faria et al., 2021a). This generalist and synanthropic (living alongside humans) behaviours also pose gulls as highly susceptible to the exposure and accumulation of contaminants such as TEs (Binkowski et al., 2024; dos Santos et al., 2024).

In particular, seabird chicks may be ideal for biomonitoring local contamination, as they are restricted to the food delivered by their parents which is often caught in the vicinities of the colony (Carravieri et al., 2017; Jouanneau et al., 2022). Moreover, the use of chicks as study models avoids the effects of the complex mix of extrinsic and intrinsic factors like age, experience, breeding status, and migration, that may confound the sources of contamination (Carravieri et al., 2014a; Finger et al., 2016). This allows to focus on narrower spatial and temporal scales, because chicks are only a few weeks old (Binkowski et al., 2021, 2024). So, the increased abundance and predictability of food sources within urban environments may ultimately act as a double-edged sword, as chicks could be more susceptible to TE contamination during their growth phase (for Hg see Ackerman et al., 2024), which corresponds to the development of the nervous system, the main target of the MeHg (Scheuhammer et al., 2007; Wolfe et al., 1998).

When working with TEs in growing chicks, however, we must consider i) chicks are fully dependent on the food delivered by their parents, ii) the concentrations of certain elements, like arsenic (As), Cd, Cu, Hg, Pb, and Se, may be influenced *a priori* by maternal transfer through egg deposition (Ackerman et al., 2016, 2017, 2020; Agusa et al., 2005; Kubota et al., 2002; Lemesle et al., 2024b; Vallverdú-Coll et al., 2015), and iii) the extent of maternal transfer can vary according to the species' breeding strategy: capital breeders (e.g., penguins) rely on energy and nutrients accumulated prior to egg formation, whereas income breeders (e.g., gulls) use resources obtained during the laying period (e.g., Whiteman et al., 2021). Thus, TE concentrations in gulls' eggs mainly reflect the female's recent diet rather than long-term body stores. For Hg, females can excrete up to 24% of body Hg load into their eggs (Ackerman et al., 2016, 2017, 2020; Lewis et al., 1993), mostly in the form of MeHg (Manceau et al., 2025). Due to its strong proteinophilic behaviour – i.e., affinity for binding to protein-rich tissues like feathers (Ajsuvakova et al., 2020) and egg albumen (Manceau et al., 2025) – MeHg is efficiently transferred from the mother to the egg, and consequently, to the embryo (Ackerman et al., 2020). High *in ovo* Hg exposure can induce embryo malposition (Herring et al., 2012) and reduce egg hatchability (Heddle et al., 2020; Herring et al., 2010). If the egg still hatches, the residual effects of *in ovo* Hg exposure may persist after hatching, reducing body condition (Santos et al., 2020), chick growth, and, in turn, survival (Bertram et al., 2025a). Besides Hg, Pb was reported to induce immunotoxicity during the embryo phase (Lee et al., 2001), during the early life of chicks (Vallverdú-Coll et al., 2015), and later disrupt the endocrine system of adult birds (Franson and Pain, 2011). This may dramatically result in poorer breeding outcomes (Burger and Gochfeld, 2001; Provencher et al., 2016; Scheuhammer, 1987). In addition, urbanisation and exposure to TEs can affect multiple physiological functions in birds beyond the endocrine system (Goodchild et al., 2022; Herrera-Dueñas et al., 2014). Elevated

concentrations of Cd, Pb, and Zn have been linked to increased heterophil/lymphocyte (H/L) ratio, an indicator of physiological stress (Krams et al., 2012), in several urban-living species (Bauerová et al., 2020; Ribeiro et al., 2022; Ushine et al., 2020). Furthermore, higher concentrations of essential and non-essential TEs have been associated with reduced haemoglobin concentration (Hb), altered erythropoiesis (Bauerová et al., 2017; Geens et al., 2010; Li et al., 2021), and genotoxic effects in adults and nestlings (Baesse et al., 2015, 2019; Quirós et al., 2008). Therefore, while dietary exposure to TEs may partially account for impaired health condition in adult birds, in growing chicks, both *in ovo* and dietary exposure may be of concern.

A good proxy for assessing *in ovo* Hg exposure could be provided by chick down feathers. This embryonic tissue shows a strong correlation with Hg concentrations measured directly in eggs (Ackerman and Eagles-Smith, 2009; Becker et al., 1994), making it a reliable, non-lethal indicator of maternal transfer of Hg. After hatching, Hg concentrations in chicks decline with age, likely due to a combination of growth dilution effect and excretion into the growing feathers (Becker et al., 1994; Wenzel et al., 1996). Once the plumage is fully developed and body mass stabilises, Hg concentrations are then expected to rise again, reflecting the dietary intake of Hg along with its negligible depuration (Ackerman et al., 2011; Bertram et al., 2025a). However, most research on maternal and dietary Hg exposure in chicks has been carried out in natural habitats (Ackerman and Eagles-Smith, 2009; Ackerman et al., 2008; Bertram et al., 2025a; Blévin et al., 2013; Sebastian et al., 2016). As a result, our understanding of Hg exposure in urban-dwelling chicks remains limited, with only a handful of studies addressing this context (e.g., Binkowski et al., 2021; dos Santos et al., 2025a; Lemesle et al., 2024c).

In the present study, we investigated the accumulation of Hg and other TEs, along with their potential effects, in yellow-legged gulls (*Larus michahellis*) breeding in the city of Porto, a large urban centre on the northwest coast of Portugal. This large gull species is known for its opportunistic behaviour while at sea, often foraging in association with fishing vessels, targeting fishery discards (Matos et al., 2018; Oro and Ruiz, 1997; Oro et al., 1997). In the urban environment, such as in coastal cities, it takes advantage of the large abundance of anthropogenic resources, such as leftovers and refuse (Lopes et al., 2021, 2022; Méndez et al., 2020), along with marine prey obtained opportunistically in fishing harbours (Pais de Faria et al., 2021a, 2021b). Therefore, we analysed Hg and 14 additional TEs in chicks of yellow-legged gulls from this urban population to characterise their exposure and assess changes along their development (only for Hg). We first measured Hg concentrations in down feathers (up to one week post-hatching) to assess *in ovo* exposure and then examined variation in blood Hg concentrations along the growing period (up to 6<sup>th</sup> week), to assess potential effects on hatching mass, early growth rate and early chick survival. Given the low blood Hg concentrations previously reported in adult gulls from Porto ( $0.98 \pm 0.71 \mu\text{g g}^{-1}$  dw; dos Santos et al., 2025b), we expect similarly low Hg concentrations in chick down feathers ( $<2 \mu\text{g g}^{-1}$  dw). Such low *in ovo* Hg exposure is unlikely to affect hatching mass, early chick survival or growth rates (but see Albert et al., 2022; Ibañez et al., 2024). Thereby, we explored associations between blood TE concentrations and physiological and health condition parameters commonly used in birds: erythrocyte sedimentation rate (ESR), Hb, H/L ratio, white blood cells (WBC) counts, immature erythrocyte (IE) counts, and erythrocyte nuclear abnormalities (ENA) (see "Immuno-haematological parameters" in Materials and Methods for detailed descriptions of each metric). Chicks with elevated concentrations of Pb, and at a given extent As, Cu, and Zn, may exhibit a compromised physiological and health condition, denoted by increased ESR and H/L ratios, and reduced Hb. On the other hand, no relationship is expected between Hg concentrations in chicks and their physiology, because Se concentrations should be sufficient to mitigate the effects of low Hg exposure (Cuvin-Aralar and Furness, 1991; Manceau et al., 2021b; Nigro and Leonzio, 1996).

## 2. Materials and methods

### 2.1. Study area and study species

This study was conducted in the city of Porto (41°08'N, 8°36'W), northwest coast of Portugal (Fig. 1). This large urban area is situated at the mouth of the Douro River, one of the largest rivers on the Iberian Peninsula. The city offers a wide variety of foraging opportunities – including the intertidal areas at the river's mouth, the river itself, landfills, refuse dumps, and urban litter – and is located approximately 7 km from a major fishing harbour in the city of Matosinhos. These features, combined with inadequate waste management, have facilitated the development of a thriving resident population of yellow-legged gulls (Pais de Faria et al., 2021a), currently estimated at 593–813 breeding pairs (Oliveira et al., 2023). This population is expected to increase in the coming years, expanding its nesting range inland, a trend already documented in other European cities (Benussi and Fraissinet, 2020).

### 2.2. Fieldwork: nest monitoring and sample collection

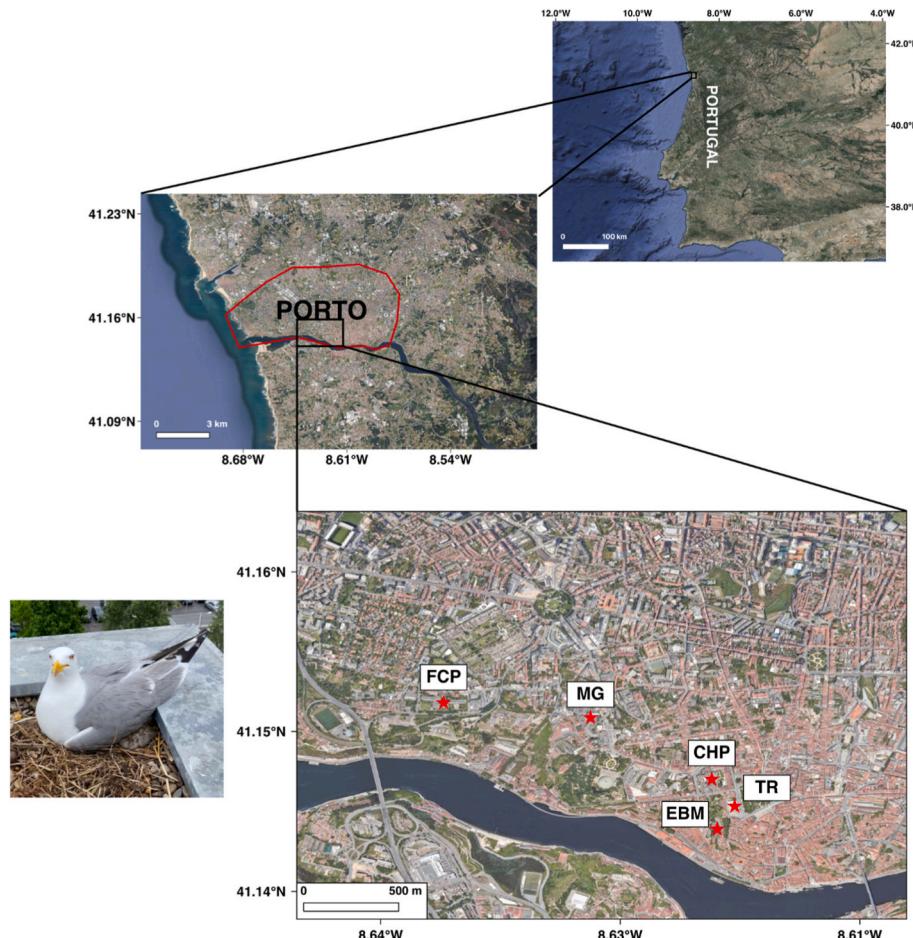
During the incubation period of 2021 and 2022, yellow-legged gull nests with a full clutch, *i.e.*, 3 eggs laid (N = 49), were marked and monitored until hatch (see Supplementary Material for details on the nest monitoring). The eggs were measured (length and width) using a calliper (to the nearest mm). Hatchlings were marked with animal marking crayon of distinct colours to identify the chick (A, B or C) with assurance. Up to 5 days old, chicks were weighted daily (to the nearest

g). Afterwards, chicks were weighed every two to four days to monitor their growth until they reached 5–6 weeks old, when their plumage was fully developed. Beyond this point, monitoring had to be stopped because handling the chicks increased the risk of them falling from the rooftops.

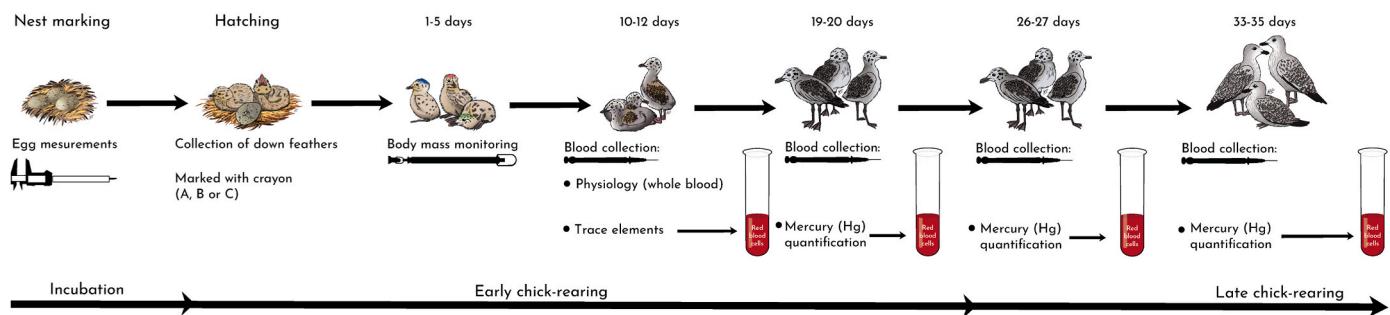
Down feathers were collected from chicks with 1–8 days old (N = 28) and used for Hg analysis. Blood was collected from the brachial vein of chicks in a weekly basis from the 2<sup>nd</sup> to the 5–6<sup>th</sup> week, obtaining a maximum of four samples *per* chick. Blood samples were centrifuged and both fractions, plasma and red blood cells (RBCs), were stored at –20 °C until analysis. RBCs from the first blood sampling were used for Hg (N = 24) and TE analyses (N = 15), while those from the following blood samplings were only used for Hg analysis (N = 72). In addition, at the first blood sampling, two capillary tubes (~150 µL) were collected to measure immuno-haematological parameters (N = 15). It is important to note that blood volumes were adjusted to chick body mass, so as not to exceed the safety values of blood sampling (Institutional Animal Care and Use Committee Standard Operating Procedure, 2016). A detailed schematic representation of the nest monitoring, sample collection, and further analyses is given in Fig. 2.

### 2.3. Down feather Hg and blood TE analyses

Down feathers were cleaned to remove surface lipids and contaminants using a 2:1 chloroform:methanol solution for 2 min, followed by two successive rinses with methanol. Feathers were then cut into small fragments and dried in an oven at 50 °C for 48 h (Blévin et al., 2013).



**Fig. 1.** Geographical location of rooftop nesting sites (red stars) of yellow-legged gulls monitored in 2021 and 2022 breeding seasons in the city of Porto, northwest Portugal. Labels stand for each site (from west to east): FCP, a faculty; MG, a residential building; CHP, a hospital; EBM, a school; TR, a court. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



**Fig. 2.** Schematic representation of the design used in this study, including the nest monitoring, sample collection, and further analyses. Chick down was collected up to 8 days old. Illustrations by Nathalie Melo (2025).

RBCs were freeze-dried and ground into a fine powder. Hg concentrations were measured in RBCs – of all samples collected from yellow-legged gull chicks along the rearing period (0.3–2.3 mg dw, N = 96) – and down feathers (0.4–0.7 mg dw, N = 28) using an advanced mercury analyser (Altec AMA 254) spectrophotometer (Bustamante et al., 2006). Blanks were run at the beginning of each set of samples, and the limit of quantification (LOQ) of the AMA was 0.1 ng. Each sample was analysed in duplicate or triplicate to ensure a relative standard deviation below 10 %. Accuracy was assessed using certified reference material (CRM, TORT-3 lobster hepatopancreas, NRC, Canada; Hg concentration:  $0.292 \pm 0.022 \mu\text{g g}^{-1}$  dw) with reference measurements performed after every 15 samples. CRM values were  $0.283 \pm 0.004 \mu\text{g g}^{-1}$  dw for RBCs (N = 11) and  $0.303 \pm 0.001 \mu\text{g g}^{-1}$  dw for down feathers (N = 3), corresponding to recovery rates of  $97.6 \pm 0.8\%$  for RBCs and  $104.5 \pm 0.4\%$  for down feathers. In parallel, a subset of chicks (N = 15) was used to analyse 14 additional TEs using freeze-dried RBCs of the first blood sample (aliquots mass ranged between 20 and 100 mg dw). Aluminium (Al), arsenic (As), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), selenium (Se), tin (Sn), strontium (Sr), and zinc (Zn) were quantified using Inductively Coupled Plasma Optical Emission Spectrometry (Agilent Technologies 5800 VDV ICP-OES), while cadmium (Cd), cobalt (Co), and lead (Pb) were quantified through ICP-Mass Spectrometry (Thermo Fisher Scientific X Series 2 ICP-MS) following Kojadinovic et al. (2011). Accuracy and reproducibility were assessed using analytical blanks and replicates of TORT-3 and DOLT-5 (Dogfish liver, NRC, Canada) along the set of samples. Recovery rates of CRMs were  $96 \pm 11\%$  for Al,  $95 \pm 6\%$  for As,  $92 \pm 2\%$  for Cd,  $88 \pm 4\%$  for Co,  $97 \pm 5\%$  for Cr,  $101 \pm 4\%$  for Cu,  $94 \pm 4\%$  for Fe,  $97 \pm 7\%$  for Mn,  $92 \pm 3\%$  for Ni,  $84 \pm 3\%$  for Pb,  $100 \pm 8\%$  for Se,  $88 \pm 7\%$  for Sn,  $90 \pm 8\%$  for Sr, and  $101 \pm 2\%$  for Zn. The limits of detection (LODs in  $\mu\text{g g}^{-1}$  dw) were 0.01 (Cd, Pb), 0.02 (Co, Sn), 0.04 (Ni), 0.06 (Sr), 0.07 (Mn), 0.1 (Al, Cu, Cr, Se), 0.2 (As), and 3.3 (Fe and Zn).

Both Hg and other TE analyses were performed at Littoral ENVironnement et Sociétés (LIENSS, CNRS-La Rochelle University) laboratory, in La Rochelle, France. The concentrations of both Hg and TEs are further given in  $\mu\text{g g}^{-1}$  dw.

#### 2.4. Immuno-haematological parameters

To assess the physiological condition and immune status of the chicks, we measured six immuno-haematological parameters commonly used in birds. Erythrocyte sedimentation rate (ESR) provides an integrative indicator of inflammation and disease processes in birds (Heylen and Matthysen, 2008; Masello and Quillfeldt, 2004). Haemoglobin concentration (Hb) reflects the oxygen-carrying capacity of the blood and is widely used as a general proxy of body condition (Minias, 2015). The H/L ratio is a well-established marker of physiological stress, increasing under acute or chronic stress responses (Davis et al., 2008; Krams et al., 2012). White blood cell (WBC) counts provide an indication of overall immune capacity, with reduced counts often associated

with immunosuppression (Nisbet et al., 2015). Immature erythrocyte (IE) counts serve as an index of erythropoietic activity and can signal anaemia-like conditions when elevated (Minias, 2015). Finally, erythrocyte nuclear abnormalities (ENA) quantify genotoxic damage and are commonly used as biomarkers of exposure to contaminants and other environmental stressors (Skarphedinsdottir et al., 2010).

All parameters were measured from the first blood sample to avoid any effects of repeated sampling and were quantified and reported following the procedures in dos Santos et al. (2024).

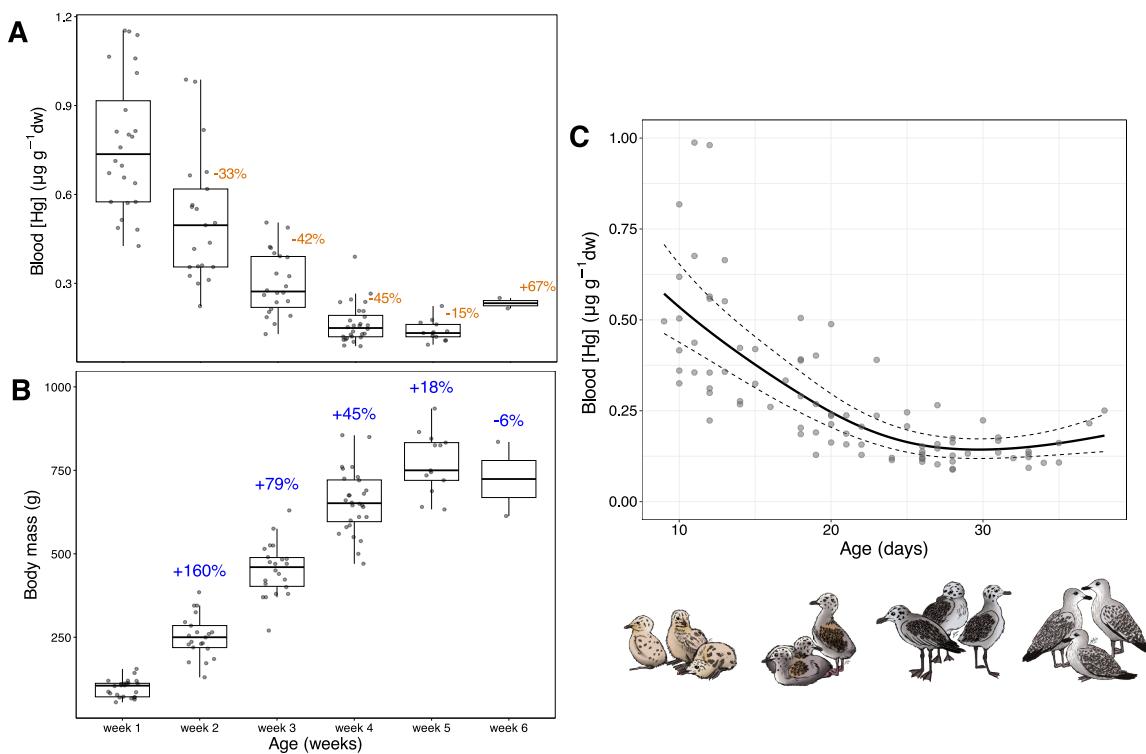
#### 2.5. Statistical analysis

Data exploration, visualisation, and statistical analysis were conducted in R v4.4.2 (R Core Team, 2024). To address our aims, we fitted regression-type models with specifications tailored to the response variable, error distribution, and relevant explanatory variables which are explained below and detailed in the Supplementary Material (Table S1). Model specification and fit were validated through residual diagnostics of the initial models to ensure assumptions of normality, homoscedasticity, and the absence of influential observations (see 'Aim 3' in Supplementary Material) were met (Zuur et al., 2009; Zuur and Ieno, 2016). These procedures were implemented using the *performance* (Lüdecke et al., 2021) and *DHARMa* (Hartig, 2022) R packages. For models including multiple continuous explanatory variables, collinearity was assessed using variance inflation factors (VIF < 2.5; Johnston et al., 2018) and variables were scaled to allow comparison of effect sizes (Zuur et al., 2009).

Model selection was based on Akaike's information criterion corrected for small sample sizes (AICc). Models were ranked by the difference in AICc between each model and the model with the lowest AICc of each set ( $\Delta\text{AICc}$ ) with the best model being selected for further inference (Burnham and Anderson, 2002). The marginal ( $R_m^2$ ) and conditional  $R_c^2$  were calculated to quantify the variance explained by fixed effects only and by the full model when including random effects, respectively (Nakagawa and Schielzeth, 2013). Parameter estimates ( $\beta \pm \text{SE}$ ), and 95% confidence intervals (CIs) of the best models were used to assess the direction and strength of effects. Variables with CIs not overlapping zero were considered to have a strong influence on the response.

##### 2.5.1. Hg concentrations as chicks aged

Linear mixed effect models (LMMs) were used to test the effect of age on log<sub>e</sub>-transformed chick blood Hg concentrations (Table S1). 'Age' was modelled as a nonlinear predictor using a natural cubic spline (ns) with three degrees of freedom (df = 3) to account for potential nonlinear relationships between age and blood Hg. The 'year' and the 'hatching sequence' were included as explanatory variables, as they may affect Hg exposure (see 'Aim 1' in the Supplementary Material), while the 'body mass' was not included due to its high collinearity with chick age (VIF > 9). Lastly, the 'nest' was included as a random effect to consider the non-independence of chicks from the same clutch (Bertram et al., 2025a).



**Fig. 3.** Changes in blood Hg concentrations and body mass as chicks aged. A) Blood Hg concentrations ( $\mu\text{g g}^{-1} \text{dw}$ ) decreasing with age (weeks), and B) body mass (g) increasing with age (weeks). Boxplots show the distribution of Hg and body mass measurements at each age class (from the 1st to the 6th week), respectively. Values are median, 25<sup>th</sup> and 75<sup>th</sup> percentiles, range (straight solid lines), and the real data points (grey). Percentages indicate the relative difference in Hg concentrations (A, orange) and in chick body mass (B, blue), respectively. C) Predicted chick blood Hg concentrations with increasing age (days). The solid line represents the predicted values extracted from the best LMM with a natural cubic spline of age ( $\text{df} = 3$ ), with nest as a random effect. Spline coefficients ( $\beta \pm \text{SE}, 95\% \text{ CI}$ ) are shown in Table 3. Dashed lines represent the 95% CI, and the points represent the raw blood Hg concentrations. Four chicks were removed from the dataset as there were only two blood samples from each one. Illustrations by Nathalie Melo (2025). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

#### 2.5.2. Effects of maternal Hg on chick hatching mass and early growth rate

Two sets of linear models (LMs) were used to test the effect of maternal Hg concentrations – inferred from chick down feather and converted in egg-equivalent Hg concentrations (see ‘Conversion of down feather Hg concentrations to egg Hg concentrations’ within ‘Aim 2’ in the Supplementary Material) – on the hatching mass (first set) and early growth rate (second set) of chicks (Table S1), calculated as explained in ‘Egg volume and chick growth calculation’ within ‘Aim 2’ in the Supplementary Material.

#### 2.5.3. Effect of TE concentrations on chick physiological and health condition

Six sets of LMs or generalised LMs (GLMs) were used to test the effect of non-collinear and no correlated TEs and Se:Hg molar ratio on each immuno-haematological parameter – ESR, Hb, H/L, WBC, IE, and ENA (see Table S1) – in chicks sampled in 2022 (see ‘Aim 3’ in the Supplementary Material).

**Table 1**

Down feather, egg-equivalent, and blood Hg concentrations ( $\mu\text{g g}^{-1} \text{dw}$ ) and body mass (g) along the nestling period (weekly measurements). Down feather Hg was converted into egg-equivalent Hg concentrations in fww (Ackerman and Eagles-Smith, 2009) and then converted into dw concentrations considering a 75% moisture content in eggs (Ackerman et al., 2013, 2024). Blood Hg concentrations were quantified from the 2<sup>nd</sup> until the 6<sup>th</sup> week old. Blood Hg concentrations for the 1st week of chicks were estimated using the equation from the best model (see Tables 2 and 3) and using nest-specific intercept ( $\mu\text{Nest}$ ) (Table S2).

N	Hg ( $\mu\text{g g}^{-1} \text{dw}$ )		Body mass (g)		
	Mean $\pm$ SD	Min-Max	Mean $\pm$ SD	Min-Max	
Down feather	28	1.45 $\pm$ 1.10	0.51–5.52	–	–
Egg-equivalent	28	0.66 $\pm$ 0.47	0.24–2.39	–	–
Blood 1 <sup>st</sup> week (4 $\pm$ 1 days old) <sup>a</sup>	24	0.77 $\pm$ 0.23	0.43–1.15	97 $\pm$ 26	55–155
Blood 2 <sup>nd</sup> week (11 $\pm$ 1 days old)	24	0.49 $\pm$ 0.21	0.22–0.99	263 $\pm$ 68	130–385
Blood 3 <sup>rd</sup> week (18 $\pm$ 2 days old)	23	0.28 $\pm$ 0.11	0.13–0.51	488 $\pm$ 83	370–680
Blood 4 <sup>th</sup> week (25 $\pm$ 2 days old)	24	0.16 $\pm$ 0.07	0.09–0.39	656 $\pm$ 94	470–855
Blood 5 <sup>th</sup> week (32 $\pm$ 2 days old)	13	0.14 $\pm$ 0.04	0.09–0.22	772 $\pm$ 91	633–935
Blood 6 <sup>th</sup> week (38 $\pm$ 1 days old)	2	0.23 $\pm$ 0.02	0.22–0.25	724 $\pm$ 157	613–835

<sup>a</sup> Estimated using the best model equation:  $\log\left(\text{BloodHg } \frac{\mu\text{g}}{\text{g}}\right) = -0.558 + \mu\text{Nest} + \beta_1\text{S1}(\text{Age}) + \beta_2\text{S2}(\text{Age}) + \beta_3\text{S3}(\text{Age})$ .

### 3. Results

#### 3.1. Hg concentrations as chicks aged

Blood Hg concentrations significantly decreased as chicks aged (Fig. 3; Table 1) – from  $0.77 \pm 0.23 \mu\text{g g}^{-1}$  dw (first week) to  $0.14 \pm 0.04 \mu\text{g g}^{-1}$  dw (fifth week) and increased afterwards (Fig. 3A) –, and inversely to chick body mass (Fig. 3B). The best model on explaining Hg concentrations in the blood of chicks included the nonlinear effect of age and a random intercept for nest (Fig. 3C and S1; Table S2), with no effect of the hatching sequence nor the year (Table 2). Age alone explained 62% of the variance ( $R_m^2 = 0.62$ ), while including the nest as random effect has increased the explained variance to 90% ( $R_c^2 = 0.90$ , Table 3).

**Table 2**

Summary outputs of model selection. This table includes the four best candidate models including the respective null models for each set. Models are ranked according to the  $\Delta\text{AICc}$  with the respective explanatory variables shown. Abbreviations:  $k$ , number of parameters; AICc, Akaike's information criterion corrected for small sample sizes;  $\Delta\text{AICc}$ , difference between AICc of the specific model and the best model;  $w_i$ , AICc weights.

Model specifications	$k$	AICc	$\Delta\text{AICc}$	$w_i$
<b>LMM ('identity' link)</b>				
<b>1) Chick blood Hg concentrations along age (N = 86)</b>				
Full model: $\log(\text{BloodHg}) \sim \text{ns}(\text{Age}, \text{df} = 3) + \text{Year} + \text{HatchSeq} + (1 \mid \text{Nest})$	5	22.87	0.00	
$\text{ns}(\text{Age}) + (1 \mid \text{Nest})$				0.85
$\text{ns}(\text{Age}) + \text{Year} (1 \mid \text{Nest})$	6	26.39	3.52	0.15
Full model	8	35.75	3.82	0.00
Null model	2	60.90	89.44	0.00
<b>LM ('identity' link)</b>				
<b>2) Chick mass at hatching in relation to maternally transferred Hg (N = 19)</b>				
Full model: $\text{HatchMass} \sim \text{EggHg}_E + \text{Year} + \text{EggVol} \times \text{HatchSeq}$	4	143.07	0.00	
$\text{Year} + \text{EggVol}$				0.83
$\text{EggHg}_E + \text{Year} + \text{EggVol}$	5	146.83	3.76	0.13
Null model	2	149.15	6.08	0.04
$\text{EggHg}_E + \text{Year} + \text{EggVol} + \text{HatchSeq}$	6	154.99	11.92	0.00
<b>LM ('identity' link)</b>				
<b>3) Chick quadratic growth coefficient in relation to maternally transferred Hg (N = 18)</b>				
Full model: $\text{QGrowth} \sim \text{EggHg}_E + \text{Year} + \text{HatchSeq}$	2	81.40	0.00	
Null model				0.63
$\text{EggHg}_E + \text{Year}$	4	83.84	2.44	0.19
$\text{EggHg}_E$	3	84.01	2.60	0.17
Full model	6	88.54	7.13	0.02
<b>LM ('identity' link)</b>				
<b>4) Chick erythrocyte sedimentation rate (ESR) (N = 12)</b>				
Full model: $\text{ESR} \sim \text{As} + \text{Cu} + \log(\text{Mn} + 2) + \log(\text{Pb} + 1) + \log(\text{Sr} + 1) + \log(\text{Se:Hg} + 2)$	3	-21.34	0.00	
$\text{As} + \log(\text{Pb} + 1)$				0.70
Null model	2	-19.20	2.14	0.24
$\text{As} + \log(\text{Pb} + 1) + \log(\text{Se:Hg} + 2)$	4	-16.43	4.91	0.06
$\text{As} + \log(\text{Pb} + 1) + \log(\text{Se:Hg} + 2) + \log(\text{Mn} + 2)$	5	-8.73	12.61	0.00
<b>GLM Poisson ('log' link)</b>				
<b>5) Chick erythrocyte nuclear abnormalities count (ENA) (N = 11)</b>				
Full model: $\text{ENA} \sim \text{As} + \text{Cu} + \log(\text{Mn} + 1) + \log(\text{Pb} + 1) + \log(\text{Sr} + 1) + \log(\text{Se:Hg} + 1)$	2	40.01	0.00	
$\log(\text{Se:Hg} + 1)$				0.45
Null model	2	41.01	0.99	0.28
$\log(\text{Se:Hg} + 1) + \text{As}$	3	41.40	1.38	0.23
$\log(\text{Se:Hg} + 1) + \text{As} + \log(\text{Sr} + 1)$	4	44.65	4.64	0.04

**Table 3**

Summary outputs of the best selected models presented in [Table 2](#). Model outputs are specified:  $\beta \pm$  standard error (SE), 95% confidence intervals (CI), and the percentage (%) of variance explained by the fixed effects only ( $R_m^2$ ) or by the full model when including random effects ( $R_c^2$ ). Whenever the null model was ranked the best model, all explanatory variables were deemed non-significant and are not presented here (see [Table S1](#)).

Response variables	Explanatory variables	$\beta \pm$ SE [95 % CI]	$R_m^2/R_c^2$
Log (Blood Hg)	Intercept	$-0.56 \pm 0.11 [-0.77 - -0.34]$	90%
	ns (Age, df = 3)1	$-1.42 \pm 0.09 [-1.60 - -1.24]$	
	ns (Age, df = 3)2	$-1.81 \pm 0.17 [-2.15 - -1.47]$	
	ns (Age, df = 3)3	$-0.84 \pm 0.11 [-1.05 - -0.63]$	
Hatching Mass	Intercept	$-12.68 \pm 25.49 [-66.73 - 41.37]$	47%
	Year (2022)	$-12.08 \pm 4.02 [-20.61 - -3.56]$	
ESR	EggVol	$0.99 \pm 0.36 [0.22 - 1.75]$	49%
	Intercept	$0.31 \pm 0.02 [0.26 - 0.36]$	
	As	$0.08 \pm 0.03 [0.02 - 0.14]$	
ENA	log (Pb + 1)	$0.08 \pm 0.03 [0.03 - 0.14]$	31% <sup>a</sup>
	Intercept	$0.77 \pm 0.21 [0.32 - 1.16]$	
	log (Se:Hg + 1)	$0.44 \pm 0.22 [0.01 - 0.89]$	

<sup>a</sup> For ENA (Poisson distribution) explained deviance is presented.

### 3.3. TEs association with chick physiological and health condition

Among the 15 TEs analysed, Al, As, Cu, Fe, Hg, Mn, Pb, Se, and Zn were quantified in 100% of blood samples ([Table S3](#)). Sr was quantified in 14 out of 15 chicks, Co in 9, while Ni was only quantified in 2 chicks. Cd, Cr, and Sn were not quantified in any sample ([Table S3](#)). Se:Hg molar ratio ranged between 7.3 and 80.9 ([Table S3](#)). The best model for explaining ESR in chicks included As and Pb as strong predictors ( $\beta \pm$  SE, As:  $0.08 \pm 0.03$ , CI: 0.02–0.14; Pb:  $0.08 \pm 0.03$ , CI: 0.03–0.14; [Table 3](#)), with both exhibiting a positive relationship with ESR ([Fig. 4](#)). Also, Se:Hg molar ratio was the only predictor included in the best model to explain ENA counts in chick blood, even though it showed a weak effect ( $0.44 \pm 0.22$ , CI: 0.01–0.89;  $\Delta AIC_{\text{Null model}} = 0.99$ ; [Tables 2 and 3](#)). Blood TE concentrations nor Se:Hg molar ratios were related to chick Hb, H/L, WBC and IE counts, as the null model was identified as the best supported model ([Table S1](#)).

## 4. Discussion

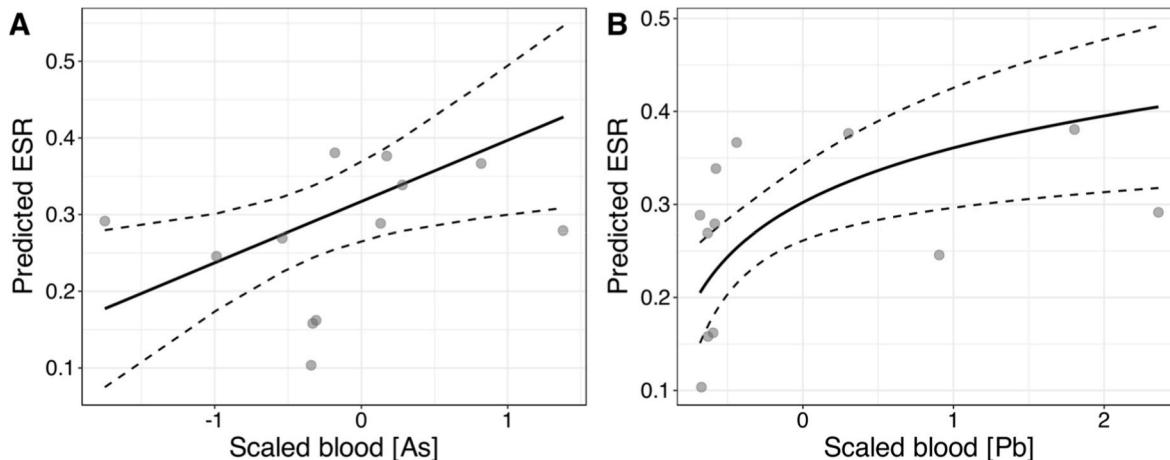
In this study, we observed that blood Hg concentrations decreased as chicks aged, with age alone explaining over half of the variation in Hg concentrations. While Se concentrations were up to 3 times lower than those usually observed in seabird chicks' blood ([Ikemoto et al., 2005; Kim et al., 2013](#); but see [Caravieri et al., 2017, 2020](#)), Pb, detected in all chicks, had concentrations exceeding reported toxicity thresholds in one third of chicks. Pb and As concentrations were associated with a faster

ESR, suggesting an increased inflammatory response and potential detrimental effects on chick physiology. Moreover, we did not detect any relationship between maternal Hg and chick body mass at hatching nor chick growth rate, which could be explained by their low contamination by Hg and sufficient Se concentrations to cope with the toxic effects of MeHg.

### 4.1. Blood Hg concentrations along chick age: mass, feather growth, and diet

The decrease of blood Hg concentrations as chicks aged can be explained by two main processes: the rapid increase of chick body mass (*i.e.*, growth dilution effect) and the continuous depuration of Hg through growing feathers ([Becker et al., 1994; Santos et al., 2020; Wenzel et al., 1996](#)). In large gull species, chicks begin growing their first plumage at around 10 days after hatching ([Sotillo et al., 2019](#)), which overlapped with the peak in blood Hg loads. As feather growth slows and body mass gain stabilises – while dietary Hg intake persists – Hg concentrations are expected to rise again, forming a U-shaped curve over time ([Ackerman et al., 2011; Bertram et al., 2025a](#)). We were not able to sample blood immediately after hatching neither to monitor chicks beyond 5–6 weeks old, as the attempt to capture them could have presented a risk of chicks falling from the rooftops.

A captive experiment carried out on lesser black-backed gull (*L. fuscus*) chicks fed with different dietary regimes – marine, mixed, and terrestrial –, revealed blood and feather Hg concentrations to be



**Fig. 4.** Predicted erythrocyte sedimentation rate with increasing blood A) As concentrations ( $\beta \pm$  SE:  $0.08 \pm 0.03$ , 95% CI: 0.02–0.14, N = 12; [Table 3](#)) and B) Pb concentrations in chicks ( $0.08 \pm 0.03$ , CI: 0.03–0.14, N = 12; [Table 3](#)). Solid lines represent the predicted lines; dashed lines represent the 95% CI, and the points represent the ESR predicted by the best model.

substantially influenced by chick diet (Santos et al., 2020). When we compared Hg concentrations in the blood of our ~30 days old chicks with those from captive chicks, we observed that our values were similar to those from chicks fed with a mixed diet (this study:  $0.14 \pm 0.04 \mu\text{g g}^{-1}$  dw,  $32 \pm 2$  days old; lesser black-backed gulls:  $0.12 \pm 0.01 \mu\text{g g}^{-1}$  dw, 30 days old; Santos et al., 2020). In Porto, young chicks are often provisioned by marine prey during their first weeks, while after that period (approx. 20 days old), adults gradually replace marine prey by anthropogenic items (up to 60 % of chick diet; Pais de Faria et al., 2021b). This shift in prey quality is often interpreted as a trade-off between chick nutritional needs (Annett and Pierotti, 1989) and near-colony resource availability, a pattern that is well-known in gulls (Lenzi et al., 2019; Méndez et al., 2020; Moreno et al., 2010). This may influence Hg accumulation along chick growth, potentially slowing Hg accumulation after feather growth.

Remarkably, nest identity also explained a substantial variance (28%) on chick blood Hg concentrations, revealing that Hg accumulation differs markedly among nests. Nest-specific patterns likely arise from several key factors: i) variation in the diet of females during egg formation (income breeders), ii) different efficiency on the maternal transfer of Hg into the eggs, or iii) parental provisioning strategies that vary in prey selection and foraging locations. In fact, individual variability and specialisation in foraging has already been documented in gulls (Maynard and Ronconi, 2018), known as a mechanism to reduce intraspecific competition (Navarro et al., 2017). Individual specialisation depends on static factors like species, sex, age or breeding stage (Ceia and Ramos, 2015; Navarro et al., 2024) but is also linked to the individual development and unique experiences that shape decisions and trade-offs, *i.e.*, learning (Phillips et al., 2017; Zango et al., 2020). Hence, different life experiences may ultimately affect individual susceptibility to anthropogenic threats, such as exposure to TEs like Hg (*e.g.*, gulls, dos Santos et al., 2025b; Jouanneau et al., 2022). Together, these findings highlight how ecological heterogeneity – even within a single population – can modulate the exposure and assimilation of contaminants in growing chicks. They also point out how Hg assessments at the colony level may overlook localised exposure, needing finer-scale monitoring of gull populations adapted to urban living.

#### 4.2. Hg toxicity risks: from the early hatching to near fledging

Evaluating the toxicity risk of a certain contaminant in growing chicks is quite challenging as concentrations can be affected by an interplay of factors beyond dietary intake. Maternal transfer (*via* egg deposition), parental provisioning rates, and chicks' metabolic rates can all modulate Hg concentrations in chicks (Ackerman et al., 2016; Kubota et al., 2002; Santos et al., 2020). Therefore, we chose to limit the assessment of risks related to Hg toxicity to two stages: i) using down feather or egg-equivalent Hg concentrations, acting as proxies of maternal transfer of Hg (Ackerman and Eagles-Smith, 2009; Becker et al., 1994; Heinz et al., 2010), and ii) using blood collected from ~30 days old chicks, as growth and feather development are already expected to have plateaued (Ackerman et al., 2011; Bertram et al., 2025a), when Hg concentrations are more representative of steady-state exposure. We restricted our comparisons to studies that reported Hg in the blood of chicks, as adult birds tend to exhibit higher concentrations due to long-term bioaccumulation and the absence of growth dilution (*e.g.*, Jouanneau et al., 2022; Lemesle et al., 2024c). To facilitate comparisons with previous studies, we assumed a moisture content of 65% in RBCs to convert Hg concentrations from ww to dw (Caravieri et al., 2022).

Chick down has been a promising tissue to correlate the embryonic exposure to Hg with the post-hatching concentrations and the potential hazardous effects of Hg in such critical stages of development (Ackerman and Eagles-Smith, 2009; Ackerman et al., 2024). In the present study, down feather Hg concentrations were low (mean  $\pm$  SD,  $1.45 \pm 1.10 \mu\text{g g}^{-1}$  dw) and similar as those reported in other gull chicks (mean range,  $0.90$ – $3.21 \mu\text{g g}^{-1}$  dw; Becker et al., 1994; Santos et al.,

2017; Szumilo-Pilarska et al., 2017; but see Santos et al., 2020; mean range,  $3.27$ – $4.08 \mu\text{g g}^{-1}$  dw), with only two chicks exhibiting concentrations outside the range of  $\pm 1$  SD. It is important to note that these chicks were siblings ( $4.61$  and  $5.52 \mu\text{g g}^{-1}$  dw), which reinforces the importance of considering nest identity when analysing the trans-generational transfer of Hg (Bertram et al., 2025a, 2025b; Lemesle et al., 2024b). Concerning the potential risk of the current Hg concentrations in chick down, we observed that with the exception of those two chicks, there is a low risk of MeHg toxicity ( $<3.90 \mu\text{g g}^{-1}$  dw, Ackerman et al., 2024).

Blood Hg concentrations observed in this study (range:  $0.09$ – $0.25 \mu\text{g g}^{-1}$  dw in chicks  $>30$  days old) were lower than those reported in chicks of the yellow-legged gull from the Western Mediterranean (mean  $\pm$  SD,  $0.50 \pm 0.58 \mu\text{g g}^{-1}$  dw; Patier et al., 2024), and in chicks of the great black-backed (*L. marinus*), herring (*L. argentatus*), and lesser black-backed gull from the French Atlantic coast (mean range,  $0.11$ – $3.10 \mu\text{g g}^{-1}$  dw; Binkowski et al., 2021; Jouanneau et al., 2022; Lemesle et al., 2024a). According to the toxicity thresholds proposed for juvenile birds, that emphasises the higher sensitivity of pre-fledged and recently fledged chicks to MeHg toxicity, our chicks were at negligible risk (no risk up to  $0.4 \mu\text{g g}^{-1}$  dw in RBCs; Ackerman et al., 2024). This finding is consistent with previous studies on adult gulls from Porto, of which 70% exhibited blood Hg concentrations below the lowest toxicity threshold (dos Santos et al., 2025b). However, this does not necessarily imply that chicks could not be affected by the current Hg concentrations. In fact, in captive lesser black-backed gull chicks with blood Hg concentrations exceeding  $0.25 \mu\text{g g}^{-1}$  dw, a decrease in energy metabolism, an increase in oxidative stress, and an elevation in lactate dehydrogenase activity have been reported (Santos et al., 2020). This further supports the notion that toxicity thresholds may vary according to species sensitivity and ecological context (Ackerman et al., 2024), according to the co-exposure to other pollutants (Korsman et al., 2016) and protective elements like Se-compounds (El Hanafi et al., 2022) or antioxidant molecules like vitamins (Sakamoto et al., 2018). Indeed, as chicks are developing these factors might be even more relevant.

#### 4.3. The protective role of Se against Hg toxicity

When assessing the toxicological risk associated with Hg, and in particular of MeHg, it is essential to consider Se (Cuvin-Aralar and Furness, 1991; Nigro and Leonzio, 1996). In this study, Se concentrations were similar to those reported in gull chicks from other colonies (Binkowski et al., 2024; Sebastian et al., 2017) and approximately half of the  $10$ – $20 \mu\text{g g}^{-1}$  dw range observed in other seabird chicks (Ikemoto et al., 2005; Kim et al., 2013; but see Caravieri et al., 2017, 2020). Low concentrations of Se may bring physiological consequences as Se is required as a cofactor for enzymes involved in fighting oxidative stress, *e.g.*, glutathione peroxidase (Gamble et al., 1997), and as it is involved in the detoxification of MeHg (Nigro and Leonzio, 1996). Specifically, the complete demethylation of one molecule of MeHg by Se involves an intermediate selenocysteinate complex – Hg-tetraselenolate ( $\text{Hg}(\text{Se})_4$ ) – that undergoes biomineralization by selenoproteins (*e.g.*, selenoprotein P) into chemically inert tiemannite ( $\text{HgSe}$ ) (Manceau et al., 2021a, 2021b). These recent studies have disclosed i) the need of a molar ratio of 4:1 between Se and Hg, rather than the conventional 1:1 stoichiometry (Koeman et al., 1973, 1975), ii) the capacity of an organism to effectively detoxify MeHg will depend on the bioavailable Se and its speciation (Manceau et al., 2021a), and iii) the potential of MeHg to disrupt Se biochemistry (Ralston and Raymond, 2018) and to modify the antioxidant function of selenoproteins and other Se-compounds, like selenoneine (El Hanafi et al., 2022). Thus, yellow-legged gull chicks from Porto seem to be protected against MeHg toxic effects as Se:Hg is above 4, mostly because of the very low Hg concentrations in the blood of chicks and not necessarily due to the high Se concentrations, as already reported for adult breeders from the same colony (dos Santos et al., 2024).

#### 4.4. TE concentration and impacts on health condition and chick growth

Trace elements such as Co, Fe, Mn, Ni, and Zn were in a similar range of concentrations as observed in the blood of Laridae and Procellariidae chicks (Binkowski et al., 2024; Carravieri et al., 2017, 2020; Kim et al., 2013; Sebastian et al., 2017), expressing their role in homeostatic processes (Walsh, 1990). Conversely, the non-detection of Cd, Cr, and Sn suggests the low exposure and/or low bioavailability of these elements in seabird chicks. In addition, Cu concentrations were up to 3-fold higher than the usual range reported in seabird blood, including other gull species (range, 0.41–2.15  $\mu\text{g g}^{-1}$  dw; Binkowski et al., 2024; Carravieri et al., 2017, 2020; Sebastian et al., 2017). However, these values were aligned to what we have observed for yellow-legged gull adults in a previous study (range, 2.13–4.92  $\mu\text{g g}^{-1}$  dw; dos Santos et al., 2024). Copper is an essential element, and thus, its concentrations are expected to remain fairly constant due to homeostatic processes (Walsh, 1990). Thus, it is possible that i) yellow-legged gull has developed tolerance to higher concentrations of Cu due to the consumption of marine invertebrates, such as shellfish and crustaceans (Alonso et al., 2015; Ariazaga et al., 2010; Munilla, 1997; Pedro et al., 2013), or ii) the optimal range of Cu concentrations for this species could be higher than for other seabird species, including other *Larus* spp. (Binkowski et al., 2024). Additionally, Al concentrations were similar to those reported in black-tailed gull chicks from South Korea (mean range: 6.38–19.57  $\mu\text{g g}^{-1}$  dw; Kim et al., 2013) and to yellow-legged gull adults from Portuguese colonies (range, 4.28–18.27  $\mu\text{g g}^{-1}$  dw; dos Santos et al., 2024). Aluminium is known to produce deleterious effects on the reproductive system and to displace Fe from transferrin, a protein that mediates the transport of Fe in the blood from absorption sites to sites of utilisation, storage, and haemoglobin degradation (Bakour et al., 2017; Yuan et al., 1989). Nevertheless, none of these elements exhibited a relationship with the immuno-haematological parameters measured in chicks.

Blood Pb concentrations (mean  $\pm$  SD,  $0.32 \pm 0.37 \mu\text{g g}^{-1}$  dw) were similar to those reported in chicks of herring and lesser black-backed gulls from the city of Le Havre in the Seine estuary, France (mean range, 0.19–0.53  $\mu\text{g g}^{-1}$  dw; Binkowski et al., 2024). The exposure to Pb has shown to be higher in urban and landfill environments (de la Casa-Resino et al., 2014; Schutten et al., 2024), due to legacy of leaded gasoline (i.e., tetraethyl Pb gasoline) and the current emissions from exhaust and non-exhaust traffic sources (Kelepertzis et al., 2020; Kayee et al., 2021; Ye et al., 2022). In fact, blood Pb concentration in chicks from Porto were up to 5-fold higher than those observed in yellow-legged gull adults from other Portuguese colonies (mean range, 0.03–0.07  $\mu\text{g g}^{-1}$  dw; dos Santos et al., 2024). Blood As concentrations were 2 to 10-fold lower (mean range, 3.48–18.10  $\mu\text{g g}^{-1}$  dw; Binkowski et al., 2024), and more similar to those reported in black-tailed gulls (*L. crassirostris*) from South Korea (mean range, 2.23–4.11  $\mu\text{g g}^{-1}$  dw; Kim et al., 2013) and laughing gulls (*Leucophaeus atricilla*) from French Guiana (mean  $\pm$  SD,  $2.70 \pm 0.80 \mu\text{g g}^{-1}$  dw; Sebastian et al., 2017). On the other hand, tracking the origin of As has been challenging in birds (Sánchez-Virosta et al., 2015), which might be even more difficult in mixed foragers like gulls. Interestingly, blood As concentrations were similar to those reported in the blood of yellow-legged gull adults from the same colony (mean  $\pm$  SD,  $1.69 \pm 2.52 \mu\text{g g}^{-1}$  dw; dos Santos et al., 2024). This was unexpected as adult seabirds often exhibit higher TE concentrations than chicks (Carravieri et al., 2020; Sebastian et al., 2017). However, in this study, blood samples used for TE analyses were collected when chicks had approximately 10–12 days old, which corresponds to the period when chicks are fed with higher amounts of marine prey (Pais de Faria et al., 2021b), potentially increasing the intake of As by chicks (Binkowski et al., 2024; Córdoba-Tovar et al., 2022).

Both Pb and As are known as potentially toxic to aquatic organisms when surpassing certain concentrations (Eisler, 1994; Neff, 1997; Vallverdú-Coll et al., 2015, 2019). Strikingly, one third of our chicks exhibited Pb concentrations above the lowest concentrations with

reported toxic effects in mallard ducklings (*Anas platyrhynchos*), i.e.,  $> 0.29 \mu\text{g g}^{-1}$  dw (Vallverdú-Coll et al., 2015). Above this concentration, ducklings exhibited lower mass at hatching, changes on the immune response, increased oxidative stress, and early-life mortality (Grace et al., 2024; Vallverdú-Coll et al., 2015). On the other hand, As toxicity thresholds in birds are still unclear, which challenge the speculation on the risk of our chicks facing the current exposure. Regardless of these uncertainties, we observed an association between Pb and As with a faster ESR. In fact, a faster ESR indicates that chicks may be under physiological stress, which may trigger an inflammatory response (Heylen and Matthysen, 2008; Johnstone et al., 2017), manifested by an increase in acute phase proteins (e.g., fibrinogen) in bloodstream, promoting blood cell aggregation (Lewis et al., 2006). Ultimately, this may suggest a general impairment on chick physiological condition and/or nutritional status (Heylen and Matthysen, 2008). Despite the lack of specificity linking ESR and TE contamination, both As and Pb are capable of producing oxidative stress, which cause damage to tissues, and can lead to increased inflammatory responses and overall impaired physiological condition in birds (Bauerová et al., 2020; Hitt et al., 2023; Ushine et al., 2020). Moreover, we observed a slight and positive effect of Se:Hg on the counts of erythrocyte nuclear abnormalities (ENAs). While a higher Se:Hg molar ratio is often indicative of a protection against MeHg toxicity (Cruz-Flores et al., 2024; Laranjeiro et al., 2025), this observation of increased ENAs at higher ratios may suggest complex interactions in developing chicks. Thus, we can hypothesise this result may reflect Se redistribution away from DNA repair enzymes towards Hg sequestration (Cebulski-Wasilewska et al., 2005) or developmental stage-specific vulnerabilities in antioxidant systems. The low Se concentrations observed in gull chicks suggest that they may be operating under Se-limited conditions. Within this context, adding Hg might create competition for limited Se reserves, which may ultimately compromise the stability of genome (Grotto et al., 2009). Nonetheless, we did not observe any relationship between maternally transferred Hg (inferred from down feather Hg), chick hatching mass and early growth rate. The low Hg exposure in urban chicks (dos Santos et al., 2025a), together with the sufficient Se concentrations to cope with MeHg toxic effects (Se: Hg  $> 4$ ) might have prevented the residual effects of maternal Hg loads (Bertram et al., 2025a; Carravieri et al., 2023; Ibañez et al., 2024).

#### 5. Conclusion

This study showed the dynamics of Hg (maternal and dietary) and other TEs in urban-dwelling gull chicks and highlights physiological consequences that may arise from TE contamination. In contrast to the overall low exposure to As and Hg, Pb concentrations in blood surpassed the threshold defined for Pb toxicity in birds. This may explain the association observed between Pb and ESR, suggesting a general disruption of the inflammatory response in urban-dwelling species. Therefore, future research should focus on establishing toxicity thresholds for TEs in seabird chicks to better integrate the observed physiological responses (for Hg see Ackerman et al., 2024). Longitudinal studies focusing on chick health, immune function, and survival in relation to TE exposure would help clarify core pathways. Moreover, the unexpected increase in ENAs frequency at higher Se:Hg ratios, highlight the need to investigate how Se allocation and MeHg detoxification processes interact under potentially Se-limited conditions, like it appears to occur in urban-dwelling gulls.

#### CRediT authorship contribution statement

**Ivo dos Santos:** Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Vitor H. Paiva:** Writing – original draft, Supervision, Resources, Funding acquisition, Conceptualization. **Ana C. Norte:** Writing – review & editing, Resources, Methodology. **Carine Churlaud:** Writing – review & editing, Methodology. **Maud Brault-Favrou:**

Writing – review & editing, Methodology. **Joana Pais de Faria:** Writing – review & editing, Methodology. **Nathalie Almeida:** Writing – review & editing, Methodology. **Ricardo Fernandes:** Writing – review & editing, Methodology. **Jaime A. Ramos:** Writing – original draft, Supervision, Resources, Funding acquisition, Conceptualization. **Paco Bustamante:** Writing – original draft, Supervision, Resources, 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.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2026.123701>.

## Data availability

Data will be made available on request.

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