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## Mercury exposure and trophic ecology of urban nesting black-legged kittiwakes from France

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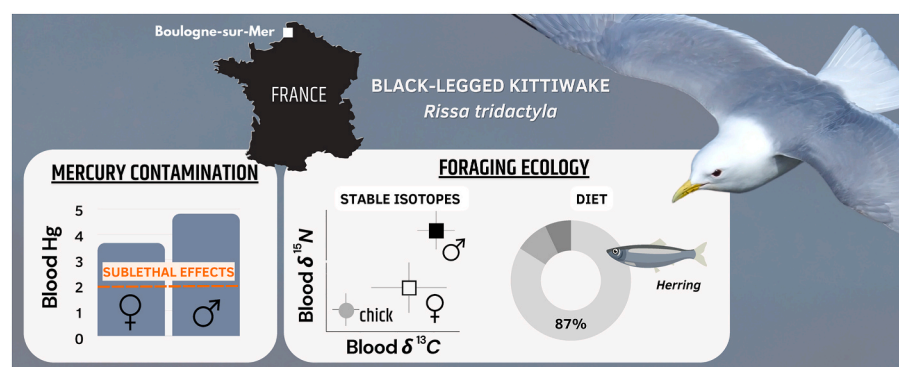
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### HIGHLIGHTS

- This is the first record of Hg levels for black-legged kittiwake in southern Europe.
- Adult blood Hg concentrations are up to three times higher than in Arctic colonies.
- 33% of adults reach concentrations categorized at moderate risk for Hg.
- Hg concentrations increased with  $\delta^{15}\text{N}$  values in adults.
- Atlantic herring and Atlantic mackerel were the main prey.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Seabirds are increasingly used as bioindicators for assessing the chemical contamination of marine ecosystems, including by mercury (Hg) worldwide. However, some geographical areas are still poorly documented, as metropolitan France that is home to 28 seabird species including the black-legged kittiwake *Rissa tridactyla*, in the part of the southern limit of the North Atlantic range of the species. Here, we investigated Hg contamination and trophic ecology of black-legged kittiwakes breeding in the harbour of Boulogne-sur-Mer, Northern France. Mean blood Hg concentration was  $4.81 \pm 1.20 \mu\text{g g}^{-1} \text{ dw}$  (dry weight),  $3.66 \pm 0.75 \mu\text{g g}^{-1} \text{ dw}$  and  $0.43 \pm 0.07 \mu\text{g g}^{-1} \text{ dw}$  for adult males, adult females, and chicks, respectively. According to Hg toxicity benchmarks for avian blood, 30% of the sampled adults were considered to be at moderate risk to Hg toxicity. Stable isotope and food analyses showed that highest  $\delta^{15}\text{N}$  values (reflecting a higher trophic position) were related to highest blood Hg concentrations in adult birds, and that Atlantic herring (*Clupea harengus*) and Atlantic mackerel (*Scomber scombrus*) were the main prey. Adult kittiwakes from Boulogne-sur-Mer showed Hg levels three times higher than those found in Arctic nesting kittiwakes, where sublethal effects have been documented. This study provides a first description of Hg contamination of black-legged kittiwakes breeding in France and calls for future ecotoxicological research to assess the vulnerability of this species in the southern part of its distribution range.

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## 1. Introduction

Mercury (Hg) is a ubiquitous metallic trace element released in the environment by natural emissions, including soil erosion, natural forest fires, and volcanism (Gworek et al., 2020; Pirrone et al., 2010). Since the Industrial Revolution, Hg anthropogenic releases have increased to exceed natural emissions due to activities such as coal burning, household waste incineration and mineral, chemical, metallurgical or electrical industries (Gworek et al., 2017, 2020; Pirrone et al., 2010; Streets et al., 2019). Despite the implementation of regulatory measures including the Minamata Convention, to reduce anthropogenic Hg releases (Kessler, 2013), this contaminant still represents a major threat to human and wildlife health (Chételat et al., 2020; Gworek et al., 2020; World Health Organization, 2022). In aquatic ecosystems, Hg is transformed by microorganisms in its toxic methylated form (methylmercury – MeHg), which is bioavailable and efficiently assimilated by living organisms (Al-Sulaiti et al., 2022; Bravo and Cosio, 2020). Hg bioaccumulates in organisms along their life and biomagnifies along food webs, leading to high concentrations in top predators and impairments of their behaviour, physiology, immune system, and reproductive success (e.g., Ackerman et al., 2024; Chastel et al., 2022; Whitney and Cristol, 2017).

Seabirds are increasingly used as bioindicators for assessing Hg contamination of marine ecosystems (Burger and Gochfeld, 2004; Elliott and Elliott, 2013; Evers et al., 2024). In this way, information on global contamination of the marine environment by Hg has improved significantly, covering temperate, tropical and polar regions (Carravieri et al., 2014a; Chastel et al., 2022; Evers et al., 2024; Gilmour et al., 2019; Sebastiano et al., 2017). However, some geographical areas are still poorly documented. This the case for metropolitan France which host 28 seabird species along its 20,000 km shoreline (GISOM, 2023), where only a few data on seabird Hg levels are available in the open literature (Binkowski et al., 2021; Jouanneau et al., 2022; Zorroza et al., 2020).

France is home to one of the most southerly breeding populations of black-legged kittiwakes (*Rissa tridactyla*, hereafter kittiwake), an abundant pelagic seabird reproducing as far north as Svalbard (Coulson, 2011; Tartu et al., 2022), and as far south as Northern Spain with a few breeding pairs (Martínez-Abraín et al., 2019). Hg concentrations in kittiwakes have been well described at the northern part of the species breeding distribution range (Iceland, Russia, and Norway including Svalbard, e.g., Albert et al., 2021, 2019; Chastel et al., 2022; Tartu et al., 2022), but data on population breeding further south, including France, are lacking.

In this study, we measured blood Hg concentrations in adults and chicks from an urban colony of kittiwakes breeding in the harbour of Boulogne-sur-Mer, Northern France, which hold a significant part of the French kittiwake population (GISOM, 2023). The first aim of this study was to provide Hg potential health risk for this kittiwake population by comparing its Hg exposure to that reported for northern kittiwake populations (Chastel et al., 2022), and by using Hg toxicity benchmarks for birds (Ackerman et al., 2016).

Foraging ecology plays a major role in Hg contamination (Ackerman et al., 2016; Anderson et al., 2009; Carravieri et al., 2014a; Jouanneau et al., 2022), therefore direct (e.g., stomach content or regurgitation analyses) or indirect (e.g., stable isotope analyses) methods give relevant and complementary information to estimate the sources of Hg contamination on short and medium terms (Barquete et al., 2013; Hobson and Clark, 1992, 1993; Lourenço et al., 2015). The second aim of this study was therefore to describe the trophic ecology of this kittiwake population, via diet sample analyses, and blood  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. As already reported for several seabird species (e.g. Binkowski et al., 2021; Jouanneau et al., 2022), we predicted that Hg concentrations should be influenced by individuals' trophic position (using  $\delta^{15}\text{N}$  as proxy), due to biomagnification of Hg in marine food webs (Lavoie et al., 2013).

## 2. Materials and methods

### 2.1. Sample collection

Fieldwork was conducted in an urban colony of kittiwake at Boulogne-sur-Mer, Northern France (50°43'35.5"N 1°35'47.1"E) on July 2020 during the chick-rearing period. In the Boulogne-sur-Mer colony, kittiwakes breed on various buildings, often at modest height, and are thus easily accessible. Twenty adults were caught with a 7 m rod with a nylon noose. Twelve chicks of approximately 20 days of age were caught by hand directly from the nest. Biometric measurements were collected for adults and chicks using a Pesola spring balance with a 10 g accuracy, a sliding caliper with a 0.1 mm accuracy for skull (head + bill), and tarsus length, and a ruler with a 1 mm accuracy for wing length. For both adults and chicks, 1 ml of blood from the brachial vein was collected with a heparinized syringe with a 25-gauge needle. For each sample, plasma and red blood cells (hereafter blood) were separated by centrifugation on return to the laboratory. Nine food sample obtained via spontaneous regurgitations from adults were also collected during the handling procedure. All samples were stored in a cooler during fieldwork and then, at  $-20\text{ }^{\circ}\text{C}$  until analyses.

### 2.2. Molecular sexing

Sex was determined by polymerase chain reaction (PCR) amplification of part of two highly conserved genes (CHD) present on the sex chromosomes (Fridolfsson and Ellegren, 1999).

### 2.3. Hg analyses

Total Hg (hereafter THg) was measured on freeze-dried and homogenised blood. For each sample, blood aliquots weighting  $0.50 \pm 0.28$  mg were analysed. THg concentrations were determined with an Advanced Mercury Analyser (®Altec AMA 254 spectrophotometer) with each sample analysed several times (at least in duplicates) until reaching a relative standard deviation below 10% (mean  $\pm$  SD:  $3.2 \pm 2.6\%$ ). The retained concentration is the mean value of these measurements. Every 20 measurements, a certified reference material for trace elements (DOLT- 5, dogfish liver, *Squalus acanthias*, Hg-certified value:  $0.44 \pm 0.18\text{ }\mu\text{g g}^{-1}$ ; National Research Council of Canada; Yang et al., 2014) was analysed under the same conditions as the samples. The recovery was  $98.4 \pm 1.0\%$  with the certified value ( $n = 5$ ,  $0.433 \pm 0.005\text{ }\mu\text{g g}^{-1}$  dw). The detection limit of the AMA was 0.1 ng. THg concentrations are expressed in  $\mu\text{g g}^{-1}$  dry weight (dw).

### 2.4. Stable isotopes analysis

The relative abundances of  $^{13}\text{C}$  and  $^{15}\text{N}$  were measured from  $0.28 \pm 0.06$  mg of blood powder weighed with a microbalance and packed in tin containers. Then, isotopes have been analysed with a continuous flow mass spectrometer (Thermo Scientific Delta V Plus®) coupled to an elemental analyzer (Thermo Scientific EA Flash®). Isotopic data were defined by the following equation:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} (\text{‰}) = ((R_{\text{sample}}/R_{\text{standard}}) - 1) \times 1000$$

where R is  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ . Isotopic data are expressed with standard notations, delta ( $\delta$ ) relative to Vienna PeeDee Belemnite and atmospheric nitrogen ( $\text{N}_2$ ) for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively. Before, during and after isotope analyses, two certified reference materials, USGS-61 (caffeine,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  certified values: 35.05 and  $-2.87\text{ ‰}$ , respectively) and USGS-63 (caffeine,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  certified values: 1.17 and  $-37.83\text{ ‰}$ , respectively) were analysed (Schimmelmann et al., 2016). The analytical precision was  $<0.10\text{ ‰}$  for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .

## 2.5. Food samples analyses

Every thawed food sample was weighed, and prey items were sorted, identified, and numbered. Fish prey were identified using otoliths, head bones (parasphenoid, maxillary, premaxillary, dentary, and articular) and other skeletal elements. Identification was carried out using our own reference collection and bibliography (Watt et al., 1997). Each species was quantified by counting caudal or proximal vertebral skeletons. Two parameters were quantified for each species: 1) the frequency of occurrence (%; *i.e.*, the proportion of regurgitates that contained this prey species), and 2) numerical abundance (%; *i.e.*, the percentage of the total number of specimens of that species in all sampled regurgitates relative to the total number of prey in all the samples).

## 2.6. Statistical analyses

First, linear models (LMs) were used to investigate the difference between adults and chicks for blood  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, and THg concentrations. For this purpose, three LMs were performed with  $\delta^{13}\text{C}$  values,  $\delta^{15}\text{N}$  values, or THg concentration after log transformation (log THg) used as the response variable, and the status (chick or adult) as the factorial explanatory variable. As adults and chicks had significant differences in their THg concentrations (Estimate [95% Confidence Interval]: 2.26 [−2.43 to −2.10]; LM:  $F_{1,30} = 756.7$ ,  $p < 0.001$ ), data from adults and chicks were considered separately in further analyses.

Second,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of individuals were used to estimate and compare the width of isotopic niche between adults (female and male) and chicks. Standard Ellipse Area (SEA) were defined to contain at least 40% of individuals with Stable Isotope Bayesian Ellipses in R package (SIBER; Jackson et al., 2011). The Bayesian approach of SEA (SEAb), which is not biased by sample size, was used to estimate isotopic niche width for adult males, adult females, and chicks. To calculate the overlap between the isotopic niches, Standard Ellipse Areas corrected for small sample size (SEAc) were used with the function maxLikOverlap (SIBER package, Jackson et al., 2011).

Third, we explored the potential main drivers of THg concentrations in kittiwakes. Prior to these analyses, Pearson correlation coefficients were calculated between  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , body condition (for adults; the residuals of the linear regression between body mass and skull length), and body mass (for chicks) to evaluate multicollinearity among explanatory variables (Supporting Information, Table S1). In chicks, we used body mass as a proxy of age (Barrett, 1996; Coulson, 2011). This parameter is an essential factor to consider, since Hg concentrations are known to change during chick growth (Ackerman et al., 2011; Monteiro and Furness, 1995). In adults, we used LMs to study THg relationship to trophic ecology and physiological parameters, including body condition, sex (factorial variable),  $\delta^{15}\text{N}$  values, and the interactions between  $\delta^{13}\text{C}$  and sex as explanatory variables. Indeed, due to correlations between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Pearson correlation,  $r = 0.63$ ;  $p < 0.01$ ; Table S1), as well as  $\delta^{13}\text{C}$  and body condition ( $r = -0.48$ ;  $p = 0.03$ ; Supporting Information (SI), Table S1), only  $\delta^{15}\text{N}$  was used for statistical analyses.

In chicks, LMs were used to investigate the relationship of THg concentrations with  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and body mass. Due to a limited number of chicks (four males and eight females), sex was not included as an explanatory variable in the analyses. However, as chicks were fed by parental regurgitations, no sexual differences were expected, which is supported by previous studies in young gulls (Binkowski et al., 2021; Jouanneau et al., 2022).

For all the models, we built a set of models ranging from the full to the null model with all possible combination of predictors and we selected the best model according to the lowest second-order Akaike's Information Criterion for small sample size (AICc, "MuMin" package, Bartoń, 2022; Buckland et al., 1997; Burnham and Anderson, 2004). If the difference between AICc values ( $\Delta\text{AICc}$ ) was higher than 2, the model with the lowest AICc was chosen. By contrast, if  $\Delta\text{AICc} < 2$ , the model with the least explanatory variable was selected.

Homoscedasticity, and normal distribution of residuals were visually assessed on the graphs of residuals versus fitted values and Q – Q plot of each initial model (Zuur et al., 2009). Validity graphs and model selection are available in the SI. All analyses were performed using R (version 4.0.2., R Core Team, 2020) and an effect was considered significant when the estimate 95% confidence interval (CI) did not include 0 or when  $\alpha < 0.05$  for Pearson correlations.

## 3. Results

### 3.1. THg levels and stable isotopes values

Blood THg concentrations were significantly higher in adults ( $4.23 \pm 1.14 \mu\text{g g}^{-1} \text{ dw}$ ) than in chicks ( $0.43 \pm 0.07 \mu\text{g g}^{-1} \text{ dw}$ ) (Fig. 1 and Table S2). Chick isotopic values were significantly different from adult values ( $\delta^{13}\text{C}$ : Estimate [95% CI]: 0.41 [−0.52 to −0.31]; LM:  $F_{1,30} = 61.24$ ;  $p < 0.001$ ;  $\delta^{15}\text{N}$ , Estimate [95% CI]: 0.39 [−0.56 to −0.22]; LM:  $F_{1,30} = 20.41$ ,  $p < 0.001$ ). Blood  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were slightly lower in chicks ( $\delta^{13}\text{C}$ :  $17.28 \pm 0.07 \text{‰}$  and  $\delta^{15}\text{N}$ :  $15.07 \pm 0.07 \text{‰}$ ) than in adults ( $\delta^{13}\text{C}$ :  $16.86 \pm 0.17 \text{‰}$  and  $\delta^{15}\text{N}$ :  $15.48 \pm 1.14 \text{‰}$ ), respectively. Chicks and adult males had areas of the isotopic niches (SEAb) relatively similar ( $0.04$  and  $0.03 \text{‰}^2$ , respectively). Adult females had a slightly larger isotopic niche ( $0.09 \text{‰}^2$ ) than chicks and adult males. The isotopic values were significantly distinct between chicks, adult males, and females, with males having the highest values for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , followed by females and then chicks (Fig. 2 and Table S3).

In chicks, blood THg concentration was negatively correlated with body mass (slope Estimate [95% CI]: 0.0010 [−0.0019 to −0.0002]; LM:  $F_{1,10} = 7.93$ ;  $p < 0.05$ , Table S4 and Fig. 3 and S1), while  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were not included in the best model. For adults, the best model explaining THg blood levels included  $\delta^{15}\text{N}$  only (slope Estimate [95% CI]: 2.31 [0.65–3.97]; LM:  $F_{1,18} = 8.58$ ;  $p < 0.01$ , Table 1 and Fig. S2) with a positive association between THg and  $\delta^{15}\text{N}$  values (Fig. 4). However,  $\Delta\text{AICc} < 2$  with model including sex could suggested that sex can also be an influential variable to explain variability in blood THg concentrations, with higher concentrations for adult males compared to females ( $4.81 \pm 1.20 \mu\text{g g}^{-1} \text{ dw}$  and  $3.66 \pm 0.75 \mu\text{g g}^{-1} \text{ dw}$ , respectively) (Estimate [95% CI]: 3.66 [2.99–4.32]; LM:  $F_{1,18} = 6.63$ ;  $p < 0.05$ , Fig. 1).

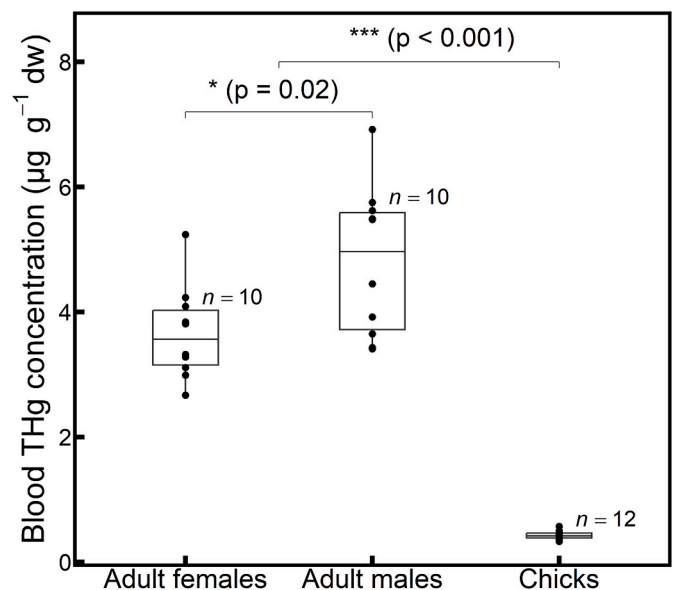
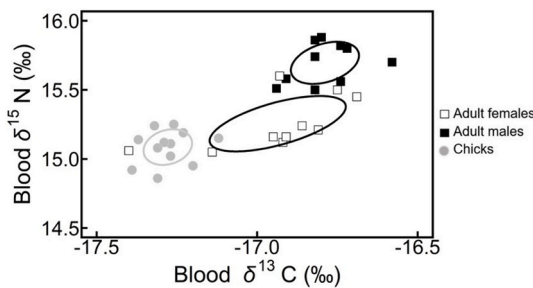
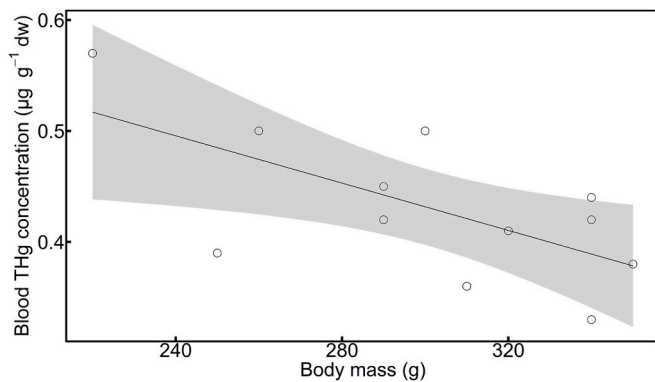


Fig. 1. Boxplots of blood THg concentrations ( $\mu\text{g g}^{-1} \text{ dw}$ ) of adults and chicks of black-legged kittiwake in Boulogne-sur-Mer; n: sampling size and p-values ( $p < 0.001$  (\*\*\*),  $< 0.05$  (\*). P-values of adult and chick differences were obtained with a logarithm transformation of THg concentrations.



**Fig. 2.** Blood  $\delta^{15}\text{N}$  versus  $\delta^{13}\text{C}$  values (‰) for adult males, adult females, and chicks of black-legged kittiwakes from Boulogne-sur-Mer. Each point is an individual value, and the ellipse indicate the Standard Ellipse Areas corrected for small sample size ( $\text{SEA}_c$ ).



**Fig. 3.** Linear regression between THg concentrations in blood ( $\mu\text{g g}^{-1} \text{ dw}$ ) and body mass (g) for black-legged kittiwake chicks of Boulogne-sur-Mer. The solid line represents the trend and the polygon represents 95% confidence intervals.  $\beta \pm \text{SE}$ : slope  $-0.001 \pm 0.000$ , intercept  $0.757 \pm 0.117$ ,  $n = 12$ .

3.2. Diet analyses

The nine food samples contained only fish. Eight fish taxa were identified, with a minimum of one taxon to four taxa per regurgitate (mean  $\pm$  SD:  $2.3 \pm 0.9$  taxa per sample). Three fish have been identified to the species level: the Atlantic herring (*Clupea harengus*, herring hereafter), Atlantic mackerel (*Scomber scombrus*, mackerel hereafter), and blue whiting (*Micromesistius poutassou*). An unidentified sandeel (*Ammodytidae* sp.), as well as four unidentified fishes were occasional prey items that occurred once. Herring was the most abundant prey item, occurring in 89% of the samples and accounting for 84% of the total number of prey (Table 2). Mackerel ranks second, occurring in 78% of the samples (9% by number). For both sexes, herring and mackerel occurred in comparable proportions of regurgitates (female: 80% and 80%; male: 100% and 75%, respectively). Similar numerical abundance

**Table 1**

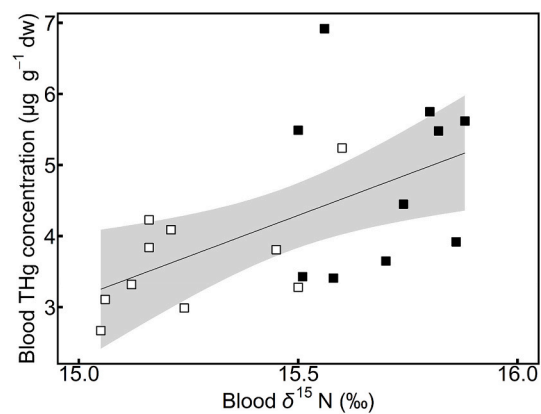
Model selections for the relationship between blood THg concentrations and foraging and biological variables – blood  $\delta^{15}\text{N}$  values, body condition, and sex – for adult black-legged kittiwakes, based on Akaike’s Information Criterion corrected for small sample sizes (AICc). The most parsimonious model is given in bold. AICcwt: Akaike’s weight; K: number of parameter;  $\Delta\text{AICc}$ : difference between the model with the smallest AICc-value and the most parsimonious model of interest.

Models	Body condition	Sex	$\delta^{15}\text{N}$	$\delta^{15}\text{N}$ : Sex	K	AICc	AICcwt		$\Delta\text{AICc}$
							n	%	
<b>Mod1</b>			X		<b>3</b>	<b>60.74</b>	<b>0.43</b>	<b>0.00</b>	
Mod2		X			3	62.26	0.20	1.52	
Mod3		X	X		4	63.58	0.10	2.84	
Mod4	X		X		4	63.79	0.09	3.05	
Mod5	X	X			4	64.13	0.08	3.40	
Mod6					2	65.73	0.04	5.00	
Mod7	X	X	X		5	66.74	0.02	6.01	
Mod8		X	X	X	5	66.89	0.02	6.16	
Mod9	X				3	68.36	0.01	7.63	
Mod10	X	X	X	X	6	70.54	0.00	9.81	

of herring (female: 82% and male: 85%, respectively) and mackerel (female: 11% and male: 9 %, respectively) were also observed for males and females.

4. Discussion

This study provides the first data on THg concentrations, trophic ecology, and diet of a southern black-legged kittiwake population. It shows a significant THg contamination in this French colony, with blood levels in adults exceeding those described for northern kittiwake populations. Our study also indicates that in adult kittiwakes, blood THg concentrations were higher for individuals with a higher trophic position (as inferred by  $\delta^{15}\text{N}$  values). Finally, our study provided an insight into the main fish prey consumed by individuals from the Boulogne-sur-Mer colony.



**Fig. 4.** Relationship between blood THg concentrations and blood  $\delta^{15}\text{N}$  values for adult black-legged kittiwakes of Boulogne-sur-Mer. White and black squares are used for female and male adults, respectively. The solid line represents the trend and the polygon represents 95% confidence intervals.  $\beta \pm \text{SE}$ : slope  $2.31 \pm 0.79$ , intercept  $-31.51 \pm 12.21$ ,  $n = 20$ .

**Table 2**

Frequency of occurrence and numbers of prey items identified from nine spontaneous regurgitates of adult black-legged kittiwakes.

Species	Occurrence		Number	
	n	%	n	%
Atlantic herring ( <i>Clupea harengus</i> )	8	88.9	71	83.5
Atlantic mackerel ( <i>Scomber scombrus</i> )	7	77.8	8	9.4
Other fishes	5	55.6	6	7.1
Total			85	100.0



4.1. Temporal integration of blood Hg and stable isotopes

Blood Hg concentrations reflect recent intakes of dietary contaminants with a relatively short half-life, which was estimated to be less than a week for chicks and up to two months for adult Cory's shearwater (*Calonectris borealis*) (Monteiro and Furness, 2001a, 2001b). Similarly, blood  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values with half-lives of less than one month provide information on a recent time window of maximum one to two months before sampling (Barquete et al., 2013; Bearhop et al., 2002; Lavoie et al., 2012; Lourenço et al., 2015). At Boulogne-sur-Mer, kittiwakes return from migration as early as February and start laying in late April and during May (pers. obs.). In different colonies, including Boulogne-sur-Mer, it was reported that they make short foraging trips, on average ~30 km from the colony, during the breeding period (Hamer et al., 2008; Harris et al., 2020; Kotzerka et al., 2010; Ponchon et al., 2017). Thus, adult blood sampling in July enables to assess the contamination and the feeding ecology on a limited spatiotemporal scale. Chicks sampled older than six days (i.e., after Hg dilution by growth of the initial contamination load transmitted by the female to the chick through the egg; Wenzel et al., 1996) also allows assessment of the local contamination and diet (Binkowski et al., 2021; Monteiro and Furness, 1995).

4.2. Hg contamination and potential health risks

Hg contamination has been documented in several populations of black-legged kittiwakes breeding at the northern part of the species

distribution. In these studies, summarized in Chastel et al. (2022), Hg has been measured from either red blood cells or whole blood samples. In kittiwakes, Hg concentrations obtained from red blood cells and whole blood are similar (Tartu et al., 2022), thus allowing direct comparison with the levels measured at Boulogne-sur-Mer. THg concentrations (average:  $4.23 \pm 1.14 \mu\text{g g}^{-1} \text{ dw}$ ) found in the red blood cells of adults black-legged kittiwake from Boulogne-sur-Mer were more than three time higher than those reported for kittiwakes breeding in the northern Atlantic (from  $0.81 \mu\text{g g}^{-1} \text{ dw}$  to  $2.38 \mu\text{g g}^{-1} \text{ dw}$ ; Arctic – Svalbard – , Barents Sea – Russia and Northern Norway – and West Greenland Sea – Iceland –, Table 3), but also in the western Atlantic (Canada). Notably, THg concentrations were two to four times lower in the well-studied Svalbard population for all measured years and all breeding stages (Blévin et al., 2017, 2018; Goutte et al., 2015; Tartu et al., 2013, 2022). Similarly, adult kittiwakes breeding in the North Pacific (Pribilof Islands, Alaska, Table 3) had lower concentrations than at Boulogne-sur-Mer. As in adult, blood THg concentrations in kittiwake chicks were higher in Boulogne-sur-Mer (mean  $\pm$  SD:  $0.43 \pm 0.07 \mu\text{g g}^{-1} \text{ dw}$  and range:  $[0.33; 0.57] \mu\text{g g}^{-1} \text{ dw}$ ) than in Greenland (mean  $\pm$  SD:  $0.14 \pm 0.05 \mu\text{g g}^{-1} \text{ dw}$ , range:  $[0.04; 0.29] \mu\text{g g}^{-1} \text{ dw}$  in whole blood converted from ww to dw; Burnham et al., 2018). However, since chicks THg concentrations change between hatching and fledging (Ackerman et al., 2011), the potential difference in age among studies makes comparisons difficult.

Current information on Hg contamination in black-legged kittiwakes are strongly biased toward Arctic and subarctic populations. It is therefore difficult to assess whether the significantly higher Hg

**Table 3**  
THg concentrations in red blood cells (RBC) of adult black-legged kittiwakes (n: sampling size, ♀- ♂: values for females and males respectively).

Study area	Year	Sampling period	n ♀- ♂	RBC THg concentrations ( $\mu\text{g g}^{-1} \text{ dw}$ )			Source
				♀ Mean $\pm$ SD	♂ Mean $\pm$ SD	Range	
<b>Northeast Atlantic</b>							
France – Boulogne-sur-Mer	2020	chick rearing	10–10	$3.66 \pm 0.53$	$4.81 \pm 1.20$	[2.66; 6.92]	present study
<b>Northwest Atlantic Subarctic</b>							
Canada – Newfoundland <sup>ab</sup>	2017	breeding period	20	1.05		[0.57; 1.71]	[1]
France – Saint-Pierre and Miquelon	2016	breeding period	6	$1.49 \pm 0.54$		[0.90; 2.23]	[2]
Canada – Gulf of St Lawrence	2006	incubation	21	$0.81 \pm 0.05$			[3]
<b>Northwest Atlantic Arctic and Baffin Sea</b>							
Canada – Baffin Island <sup>ab</sup>	2018	breeding period	14	1.48		[0.86; 1.86]	[1]
Greenland – Kippaku <sup>ab</sup>	2017	breeding period	25	0.61		[0.42; 0.90]	[1]
Greenland – Ritenbank <sup>ab</sup>	2015	breeding period	10	0.81		[0.62; 1.09]	[1]
Greenland – Qaanaaq <sup>ab</sup>	2015	breeding period	18	0.95		[0.38; 1.81]	[1]
Greenland – multisites <sup>ab</sup>	2010–2011	post-laying	100	$1.29 \pm 0.33$		[0.57; 2.48]	[4]
<b>Barents Sea</b>							
Russia – Franz Josef Land <sup>ab</sup>	2015–2017	breeding period	48	1.04		[0.48; 1.57]	[1]
Norway – Hornøya <sup>ab</sup>	2017	breeding period	15	1.14		[0.71; 2.24]	[1]
<b>East Greenland and Norwegian Sea (North Norway)</b>							
Svalbard – Krykkjefjellet <sup>a</sup>	2000–2019 <sup>c</sup>	chick rearing	594	$1.14 \pm 0.27$			[5]
Svalbard – Krykkjefjellet <sup>a</sup>	2000–2016 <sup>c</sup>	incubation	211	$1.67 \pm 0.25$			[5]
Svalbard – Krykkjefjellet <sup>a</sup>	2008, 2009, 2011 & 2016	pre-laying	346	$1.97 \pm 0.17$			[5]
Svalbard – Krykkjefjellet	2015–2018	breeding period	132	1.29		[0.38; 2.62]	[1]
Svalbard – Krykkjefjellet	2015	incubation	20–20	$1.43 \pm 0.38$	$2.00 \pm 0.59$		[6]
Svalbard – Krykkjefjellet	2012	chick rearing	22–22	$0.89 \pm 0.05$	$1.14 \pm 0.07$	[0.51; 1.64]	[7]
Svalbard – Krykkjefjellet	2009	pre-laying	52	$2.01 \pm 0.41$	$2.33 \pm 0.55$		[8]
Svalbard – Krykkjefjellet	2008	pre-laying	53	$1.97 \pm 0.44$	$2.06 \pm 0.44$		[8]
<b>West Greenland Sea</b>							
Greenland – Scoresby Sund <sup>ab</sup>	2017	breeding period	25	0.9		[0.62; 1.95]	[1]
Iceland <sup>ab</sup>	2015–2018	breeding period	23	1.33		[0.48; 3.33]	[1]
<b>Pacific</b>							
USA – Pribilofs Islands <sup>ab</sup>	2017	breeding period	8	2.38		[1.48; 3.14]	[1]
Western North America <sup>ab</sup>	2016–2017	breeding period	41	1.29		[0.18; 2.38]	[1]
USA – St-Lawrence Island <sup>ab</sup>	2016–2017	breeding period	16	1.14		[0.29; 1.71]	[1]

<sup>a</sup> THg sampled on whole blood.

<sup>b</sup> Hg converted from wet weight to dry weight using 79 % moisture content for blood (Eagles-Smith et al., 2008).

<sup>c</sup> no data for 2010. [1] Chastel et al. (2022); [2] Chastel et al. *unpublished data*; [3] Lavoie et al. (2010); [4] Burnham et al. (2018); [5] Tartu et al. (2022); [6] Blévin et al. (2018); [7] Blévin et al. (2017) [8] Goutte et al. (2015).

concentrations found south of the species' range reflect a latitudinal variation in Hg contamination, as reported in the southern hemisphere (Carravieri et al., 2020). Indeed, the high Hg levels found in kittiwakes from Boulogne-sur-Mer could also reflect local contamination originating from the strong industrial history of the English Channel area that led to high local emissions of metallic elements during the last century. The production of coal and textiles, as well as metallurgical and chemical factories were mainly concentrated near the ports of Calais, Dunkerque, and Boulogne-sur-Mer (Francescangeli et al., 2016; Lottin, 2014). Increasing numbers of gulls, including kittiwakes, are establishing urban breeding colonies on buildings (Méndez et al., 2020), including at Boulogne-sur-Mer (GISOM, 2023; Legroux, 2019). Urban trophic resources, including human-related ones, can influence contamination burden in *Larus* gull species (Thorne et al., 2021). For kittiwakes, it is unlikely that urban resources influence Hg levels in Boulogne-sur-Mer because kittiwakes only use marine resources caught at sea as confirmed by the collected regurgitates (Goutte et al., 2015; Vihtakari et al., 2018; this study). In addition, studies on bivalves (Briant et al., 2017) did not report hotspots in English Channel, suggesting further investigations on the Boulogne-sur-Mer colony and pelagic fish that compose the diet of kittiwakes, to explain these high Hg concentrations.

According to Hg toxicity benchmarks for bird blood (Ackerman et al., 2016), all adult female kittiwakes from Boulogne-sur-Mer, except one, were categorized at low risk ( $0.95\text{--}4.76\ \mu\text{g g}^{-1}$ , converted from  $0.20\ \mu\text{g g}^{-1}$  ww with a moisture percentage of 79%, Eagles-Smith et al., 2008). One female ( $5.24\ \mu\text{g g}^{-1}$  dw) was classified at "moderate risk" ( $4.76\text{--}14.27\ \mu\text{g g}^{-1}$  dw converted). In males, 50% of the sampled individuals were at "moderate risk", while the other 50% were classified as "low risk". However, although concentrations were into the "low risk" group, they were four times higher than those of a kittiwake colony from Svalbard where sublethal effects of Hg on the reproduction have been documented (Chastel et al., 2022). Indeed, in this Svalbard population, blood concentrations of  $\sim 2\ \mu\text{g g}^{-1}$  dw during the pre-breeding period were reported to influence the reproductive status with skipped reproduction related to changes in Hg-impaired endocrine secretion (Tartu et al., 2013). The lowest contaminated adult ( $2.67\ \mu\text{g g}^{-1}$  dw) in the present study had a higher concentration than this threshold, and the mean value for adults was more than two-times higher. Moreover, as found in Svalbard kittiwakes, blood THg concentrations decrease during the breeding period (Tartu et al., 2022), being the highest during the pre-laying stage (courting and nest building). This suggests that Hg concentrations in Boulogne-sur-Mer during the pre-laying period could have been even higher than those reported during the chick-rearing period. In addition, Hg concentrations in males at Boulogne-sur-Mer are on average three times higher than in kittiwakes from Svalbard, where such blood concentrations affect parental hormone secretion (prolactin) and hatching success (Goutte et al., 2015; Tartu et al., 2016). Such elevated Hg concentrations may therefore pose a threat to the population dynamic of the kittiwake population at Boulogne-sur-Mer, although this population has sharply increased between 2000 and 2021 (Gallien, 2016; GISOM, 2023). Favorable local foraging conditions combined with low predation rate at the colony may have somewhat mitigated the toxic effects of Hg. Furthermore, additional ecotoxicological studies as well as measurement of the blood levels of selenium – an oligo element which may bind to Hg, lowering its toxicity (Cruz-Flores et al., 2024; Ikemoto et al., 2004; Manceau et al., 2021) – are required to better assess potential health risks to this black-legged kittiwake colony.

Regarding potential risks for health in chicks, blood THg concentrations at Boulogne-sur-Mer were considered as below the "low risk" threshold established in adults (Ackerman et al., 2016). However, toxicity levels established in adults are difficult to apply on chicks due to physiological differences. Moreover, few studies have examined the effects of Hg on chick physiology, making the assessment of toxicity risks difficult. However, as for adults, blood Hg concentrations in kittiwake

chicks from Boulogne-sur-Mer are about three times higher than those observed in kittiwake chicks from Greenland (Burnham et al., 2018).

#### 4.3. Factors influencing blood Hg concentrations

Higher THg in adults than in chicks is commonly observed in other kittiwake colonies (Burnham et al., 2018) or seabird species (Carravieri et al., 2014a; Jouanneau et al., 2022; Sebastiano et al., 2017). Hg concentrations measured in blood reflects recent exposure of up to two months due to rapid turnover of the blood cells (Monteiro and Furness, 2001a), the shorter time window of exposure of 15-day-old chicks may, therefore, explain the observed difference with adults. Distinct isotopic niches between adults (for both sexes) and chicks, without overlap, also suggest that adults and chicks consumed prey from different trophic levels. Although we observed a significant difference in  $\delta^{13}\text{C}$  values, the natural environmental variability in  $\delta^{13}\text{C}$  baseline value (Barnes and Jennings, 2009), as well as the low variability in the range of observed  $\delta^{13}\text{C}$  values (range:  $[-17.40\text{--}16.58]\ \text{‰}$ ) do not support a clear distinction in the choice of foraging areas between adult and chick diet, and therefore cannot explain the differences found in THg levels between both ages. However, chicks had also lower  $\delta^{15}\text{N}$  values compared to adults suggesting that chicks are fed with prey of lower trophic position, which could also explain the higher THg levels in adults than in chicks.

In the present study, we found that THg concentrations were not related with stable isotope values in kittiwake chicks, contrarily to what was previously reported for chicks of other larid species (Binkowski et al., 2021; Jouanneau et al., 2022), in which higher trophic position was associated with higher Hg contamination. A specialist diet with low prey diversity to feed the chick can induce a restricted isotopic niche which could be insufficient to detect associations between Hg and isotopic tracers. This hypothesis is supported by the homogeneity of prey identified in adult regurgitates (probably intended to feed the chicks). Moreover, Hg dilution during chick growth could blur its associations with isotope values. This hypothesis is supported by the significant decrease of THg concentrations with body mass (a proxy of chick growth) confirming a THg dilution, possibly by growth in size and mobilization in growing feathers (Ackerman et al., 2011; Wenzel et al., 1996).

In adults, the positive correlation between THg concentrations and  $\delta^{15}\text{N}$  values suggests increasing Hg contamination with increasing trophic level of prey (Fig. 4), a well-known consequence of bio-magnification processes (review in Kidd et al., 2011). This is corroborated by several studies on seabirds, including kittiwakes (Anderson et al., 2009; Jouanneau et al., 2022; Lavoie et al., 2010; Øverjordet et al., 2015). As  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values are correlated, we also supposed a positive relationship between THg and  $\delta^{13}\text{C}$  in adults. However,  $\delta^{13}\text{C}$  values presented low individual variability (from  $-17.70$  to  $-16.58\ \text{‰}$ ) that cannot biologically explain the variability in THg concentrations. Additional studies on adult feeding ecology including spatial use of the marine ecosystem and prey Hg concentrations would provide valuable insights into the origin of contamination.

We found that sex was not the main factor explaining the variability of Hg but could be an influential variable. This is consistent with previously reported sex differences in Hg concentrations in several seabird species (Becker et al., 2002; Robinson et al., 2012). Since egg-laying is a significant excretion pathway of Hg for female birds, it has often been claimed as a major explanation for their lower contaminant levels (Mills et al., 2022), although its prevalence is still discussed (Bustamante et al., 2016; Hitchcock et al., 2019; Robinson et al., 2012). Another hypothesis to explain sex difference in Hg exposure is related to different foraging areas between sexes (Bustamante et al., 2016; Carravieri et al., 2014b; Hitchcock et al., 2019). In the present study, males and females had non-overlapping trophic niches, with males consuming prey of higher trophic levels compared to females, suggesting different foraging ecology as previously reported in chick-rearing kittiwakes (Tartu et al., 2022). Nonetheless, analyses of regurgitates could not highlight any

clear distinction in male and female diet, although the sample size was low and did not consider seasonal variability in feeding ecology.

## 5. Conclusion

This study provides the first description of Hg contamination of black-legged kittiwakes breeding in a colony in the southernmost part of the European breeding area. In adults, THg concentrations were associated to a high trophic position. Adult and chick exhibited high Hg concentrations compared to colonies of higher latitude in Europe. If the consequences of the measured THg concentrations are difficult to assess for chicks, in adults, all individuals exceeded values known to affect reproduction in this species. Given the elevated concentrations measured in this colony, associated with the specific constraints of breeding close to the limits of the breeding area, we suggest further studies to evaluate the risks such THg concentrations may induce on this kittiwake population.

## CRedit authorship contribution statement

**Prescillia Lemesle:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **William Jouanneau:** Writing – review & editing, Supervision, Investigation, Formal analysis. **Yves Cherel:** Writing – review & editing, Investigation. **Nathan Legroux:** Writing – review & editing, Investigation. **Alain Ward:** Writing – review & editing, Investigation. **Paco Bustamante:** Writing – review & editing, Supervision, Resources. **Olivier Chastel:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

## Declaration of competing interest

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

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2024.142813>.

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