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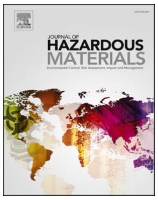

The role of seabird foraging strategies on the uptake of mercury: A case study using gulls and shearwaters from the Portuguese coast

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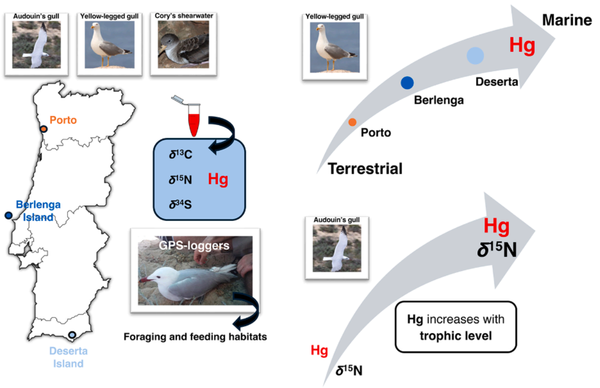
HIGHLIGHTS

- Hg, trophic ecology, and foraging habitats were studied in gulls and shearwaters.
- 37 % of adults were at moderate risk of Hg-related toxic effects.
- Stable isotopes-Hg relation varied highly among breeding colonies and study species.
- Hg concentrations increased with $\delta^{15}N$ values in gulls, but not in Cory's shearwaters.
- Greater use of terrestrial habitats may reduce Hg concentrations in gulls.

GRAPHICAL ABSTRACT



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ABSTRACT

Mercury (Hg) is a non-essential element that bioaccumulates and biomagnifies in food webs through site-specific biogeochemical processes. Seabirds are valuable bioindicators of Hg contamination, yet certain regions, like the Portuguese coast, remain underrepresented. This study measured Hg concentrations in the blood of yellow-legged gulls (*Larus michahellis*), Audouin's gulls (*Ichthyaetus audouinii*), and Cory's shearwaters (*Calonectris borealis*) breeding along the Portuguese coastline. The influence of foraging ecology on Hg contamination was investigated using stable isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) along with GPS-loggers. Thus, 52 % of the adults were at low risk (0.95–4.8 μg g^{-1} dry weight, dw), while 37 % were at moderate risk of Hg toxicity (>4.8 μg g^{-1} dw). The

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highest trophic positions (indicated by δ^{15}N values) were associated with higher Hg concentrations, particularly in gulls from Deserta Island. Yellow-legged gulls foraging in terrestrial habitats (inferred from lower δ^{13}C and δ^{34}S values and GPS data) generally exhibited lower Hg concentrations, although patterns varied between regions. Gulls from Deserta Island had elevated Hg concentrations, likely because they feed on discarded demersal fish. In contrast, those from Porto showed some of the lowest concentrations, likely because they fed on terrestrial food. However, other factors not evaluated here, like age, sex, or even phylogeny could have also influenced Hg uptake and bioaccumulation and should not be disregarded in future research. This study highlights the critical role of foraging strategies in Hg contamination and stresses the importance of estimate food web-specific baseline isotopic composition to better understand how these differences may impact Hg trophic transfer.

1. Introduction

Mercury (Hg) is one of the most well-studied elements worldwide, mostly because of its toxicity to wildlife and humans. Anthropogenic Hg releases began to rise dramatically in the 19th century with the onset of its use for gold mining extraction and fossil fuel combustion, which led to its rapid global dispersion through atmospheric transport, reaching even the most remote places such as the polar regions [1–4]. Once deposited in the marine environment, Hg is methylated mostly by microorganisms into one of its most toxic organic forms, methyl-Hg (MeHg) [5,6]. MeHg is reported to bioaccumulate in living organisms (increasing its concentrations over time in the tissues), and to biomagnify within food webs (increase its concentrations at each trophic level) [7,8]. This poses severe risks to humans and wildlife due to MeHg neurotoxic properties, which may impair reproduction, behaviour, and survival [9,10].

Top predators, such as seabirds, may be often exposed to high concentrations of Hg, which is mainly acquired *via* the diet [11–13]. The diversity of seabird feeding strategies [14,15] has shown a role in driving the exposure to Hg [13,16,17]. This has allowed the use of seabird species, or populations, as proxies of the health and quality of the foraging habitats and resource use [2,18,19]. In addition, substantial inter-individual variation in foraging behaviour, including habitat use and diet, may influence Hg exposure [14,20]. Thus, characterising foraging ecology at the individual level is crucial for understanding patterns of Hg contamination both within and among species. Higher Hg concentrations are closely associated with increased consumption of marine prey and utilisation of marine habitats, rather than terrestrial resources, as observed in gulls [16,17,21,22]. However, in strict marine foragers such as shearwaters, this pattern might be difficult to observe mostly due to their narrower trophic niche [23,24], *i.e.,* lower diversity of prey resources and reliance on the same food web baseline, which may result in relatively similar Hg concentrations among the individuals from the same colony.

Tracking devices, such as global positioning system (GPS) loggers and GPS/GSM transmitters – global system for mobile communication – enabled the monitoring of individuals' foraging movements at a fine scale and during longer periods, providing more details on habitat use [25]. In addition, the use of stable isotopes as proxies of the trophic niche *e.g.*, [26], has become a powerful tool to unravel annual [27], sexual [28], and inter-colony differences [29] in seabirds, providing integrative pictures of the assimilated diets and foraging habitats [30, 31]. Nitrogen isotopic composition (δ^{15}N; ^{15}N/^{14}N) is often used as a proxy of trophic position because of its predictable stepwise increase of 2 – 5 ‰ per trophic level [32]. Carbon (δ^{13}C; ^{13}C/^{12}C) and sulphur isotopic composition (δ^{34}S; ^{34}S/^{32}S) are used as proxies of the foraging habitats [33,34]. More specifically, δ^{15}N values are usually higher in marine rather than in terrestrial prey and its consumers [35]. On the other hand, δ^{13}C values decrease from inshore and benthic to offshore and pelagic habitats, respectively [26,36], while δ^{34}S values increase from terrestrial to marine habitats [16,31]. Thus, the combined use of tracking devices, stable isotopes, and contaminants may enable a more comprehensive view of the individuals' feeding strategies, with the power to evidence resource partitioning [37,38], and to reveal patterns of Hg contamination [16,39–42]. However, the combined use of telemetry, isotopic composition, and contaminant concentrations in bird tissues requires a thorough understanding of the turnover rates of contaminants and of tissues themselves, even when comparing contaminant concentrations between regions [43]. For instance, blood Hg concentrations provide a snapshot of recent Hg exposure due to its short half-life of up to two months [44], which is in line with blood δ^{13}C and δ^{15}N turnover rates of up to one month [45–47]. This supports the combined use of Hg and stable isotopes in blood for ecotoxicological assessments, particularly during the breeding period, as blood sampling is minimally invasive and effectively reflects short-term dietary exposure to Hg [43].

In the Iberian Peninsula, contamination of adult seabirds by Hg has been extensively documented in gulls *e.g.*, [48–50], but less in shearwaters [23,51,52]. Although, only a few studies used blood as the sampling tissue and have attempted to integrate these data with isotopic information [23,53,54]. None of these previous studies attempted to explore cause-effect relationships between tracking data and adults' main feeding habits with Hg concentrations, as carried out for sympatric gulls breeding on the Bay of Biscay, France [16]. Thus, our understanding of the impact of trophic ecology and feeding habitats on Hg concentrations remains limited for coastal and oceanic seabird populations breeding on the Iberian Peninsula.

Here, we investigated Hg concentrations in the blood of yellow-legged gulls (*Larus michahellis*), Audouin's gulls (*Ichthyaetus audouinii*), and Cory's shearwaters (*Calonectris borealis*) breeding on five colonies located in the western and southern coasts of Portugal. Despite the high blood Hg concentrations found in a previous study for Audouin's and yellow-legged gulls, Hg alone was not strong enough to depict either species' differences [53]. Instead, trophic position and feeding habitats have partly explained the variance among species and colonies, underlining the importance of including stable isotopes as predictors of Hg contamination in these populations [53]. Thus, we were particularly interested in i) describing the blood Hg concentrations in these species during the breeding season and to identify whether differences in Hg concentrations were related to the different use of resources, inferred from stable isotope analysis (δ^{13}C, δ^{15}N, and δ^{34}S); ii) investigate whether fine-scale GPS tracking may further clarify about the variation in adults' blood Hg concentrations.

Given the known dietary differences among the three species and the ecological contexts of each colony [55–57], we expected a high inter- and intra-specific variation in blood Hg concentrations. We predicted that adults occupying higher trophic positions (reflected by elevated δ^{15}N values) or those foraging in marine environments (reflected by higher δ^{13}C and δ^{34}S values and by GPS tracking data) would exhibit higher blood Hg concentrations as demonstrated by recent studies [16, 21,22,54]. However, we stress that these patterns might be more evident for the yellow-legged gull and less marked for Audouin's gull or Cory's shearwater. This is because the latter two species exhibit more specialist diets than yellow-legged gulls [24,56], which could induce narrower isotopic niches, thus being insufficient to detect the usual association between Hg concentrations and stable isotope values [12,16,21,58,59].

2. Materials and methods

2.1. Study sites and study species

From 2021 to 2023, incubating yellow-legged gulls (hereafter YLG) and Audouin's gulls (hereafter AG), and chick-rearing Cory's shearwaters (hereafter CS) were captured at three breeding locations along the western and southern coasts of Portugal: the city of Porto (41°08'N, 8°36'W; YLG, N = 29), Berlenga Island (39°24'N, 9°30'W; YLG, N = 30; CS, N = 30), and Deserta Island (36°57'N, 7°53'W; YLG, N = 58; AG, N = 49). The YLG breeds in all locations, although in quite different population densities [60], while AG only breeds at Deserta Island and CS at Berlenga Island (a total of five breeding colonies). Briefly, Porto is a large city with a thriving population of YLGs that takes advantage of predictable anthropogenic food sources such as urban waste and landfills [57,61]. Berlenga is a small neritic island located ~11 km from Peniche, a small city with a medium-sized fishing harbour. Here, the population of YLGs takes advantage of both marine and terrestrial food sources, *e.g.,* fishery discards and waste [38]. However, their diet has been consistently composed of considerable amounts of Henslow's swimming crab (*Polybius henslowii*) [56]. Lastly, Deserta is an uninhabited sandy island placed within the lagoon system of Ria Formosa, Algarve, close to the large fishing harbour of Olhão. Here, the population of YLGs exhibits a pronounced mixed-foraging strategy, taking advantage of landfills and fishery discards from trawlers [38,56,62]. In contrast, AG and CS are strictly marine foragers, despite exploiting different marine habitats: AG predominantly forage within the continental shelf and can take advantage of fishery discards [56], while CS may use both coastal marine (*i.e.,* neritic regions) and oceanic habitats, which drives quite distinct diets [24,55].

2.2. Fieldwork and sample collection

Incubating gulls with a full-size clutch were caught using a nest trap, while shearwaters were caught by hand at night when adults returned to the colony. Adult breeders were ringed, weighed (± 5 g, using a Pesola Spring© balance), wing length measured (± 1 mm, using a metal ruler) and tarsus length measured (± 1 mm, using a calliper). About 1 mL of blood was sampled using heparinized syringes coupled to a 25-G needle and stored in a cool box. Blood was then centrifuged to separate plasma from blood cells and both fractions were stored at −20 °C. Blood cells were then used to quantify total Hg (THg) concentrations and for stable isotope analysis.

A subset of the captured adults (YLG: N = 43; AG: N = 22; CS: N = 24) were equipped with GPS-loggers (CatLog; Perthold Engineering) carefully attached to the four central tail feathers with TESA© tape. The total mass of the device together with the tape weighed approximately 15 g (*i.e.,* about 2.3 % of the body mass of AG, the smallest species in this study). Each logger was programmed to record positions every 5 min and started to be retrieved one week after the deployment. For the urban-dweller YLG population (N = 12), and additional YLGs and AGs breeding on Deserta Island (N = 8 and N = 6, respectively), spatial movements were tracked using high-precision GPS transmitters (GPS/GSM Flyway-18, Movetech Telemetry). Devices were carefully attached to individual's back with four Teflon strings tied with dental floss (to allow breakage and release in a few months) during the incubation period and programmed to record positioning information every 30 min (for the urban dwellers) and 5 min (for YLGs and AGs breeding on Deserta Island) data continuously during several months (Table S1). It is important to denote that tracking data from the urban-dweller YLG population was collected from 2018 to 2020, and thus, it does not correspond to the same individuals sampled for blood either from the same breeding seasons. Therefore, tracking data collected from urban-dwelling YLGs was only used to provide an overall picture of the habitat use of this population.

All samples were collected in compliance with the European and Portuguese animal welfare regulations (EU Directive 2010/63/EU and Portuguese Decree-Law no.113/2013). The necessary licenses for sampling, 517/2021/CAPT, 350/2022/CAPT, and 316/2023/CAPT were granted by Instituto da Conservação da Natureza e Florestas (ICNF), the authorised governing body in Portugal.

2.3. THg analysis

Blood cells (hereafter 'blood') were freeze-dried and grounded to a homogenised powder prior to THg analysis. THg concentrations (N = 196, 0.3–1.0 mg dry weight, dw) were quantified using an advanced mercury analyser (Altec AMA 254) spectrophotometer [63] at the LIENSs, La Rochelle University. Each sample was analysed in duplicate or triplicate until the relative standard deviation among measurements was $< 10\,\%$ to validate the determination of its THg concentration. Certified Reference Material (CRM) TORT-3 lobster hepatopancreas (Hg certified concentration: $0.292 \pm 0.022\ \mu g\ g^{-1}$ dw) was used to check the accuracy of the method at each 15 samples. Mean concentration of the CRM was $0.288 \pm 0.004\ \mu g\ g^{-1}$ dw (N = 20), corresponding to a recovery rate of $99.2 \pm 1.5\,\%$. Blanks were run at the beginning of each set of samples. The limit of detection of the AMA used in this study was 0.1 ng and THg concentrations are further expressed as $\mu g\ g^{-1}$ dw.

2.4. Stable isotope analysis

Stable isotope analysis of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ was carried out using freeze-dried blood. Samples were weighed (between 0.2 and 0.4 mg dw for carbon and nitrogen, and 0.6–0.8 mg dw for sulphur) with a microbalance and packed into tin capsules for combustion. As AG and CS display a strictly marine foraging strategy [24,62], the sulphur isotopic composition was only measured in YLGs to assess the reliance on marine *vs.* terrestrial resources by adults breeding on the three colonies. Relative abundances of the three isotopes were measured using a continuous flow mass spectrometer (Delta V Plus with a Conflo IV interface, Thermo Scientific, Bremen, Germany) coupled to an elemental analyser ($\delta^{13}C$-$\delta^{15}N$: Thermo Scientific EA 1112; $\delta^{34}S$: Thermo Scientific EA IsoLink) at the laboratory LIENSs. Results were expressed using the delta (δ) notation and computed using the following equation: $\delta X = [(R_{Sample}/R_{Standard}) - 1] \times 1000$, where X stands by ^{13}C, ^{15}N, or ^{34}S and R being the respective ratio. Standard values correspond to Vienna PeeDee Belemnite, atmospheric N_2, and Vienna Canyon Diablo Troilite for carbon, nitrogen, and sulphur, respectively. Replicate measurements of reference materials ($\delta^{13}C$ and $\delta^{15}N$, USGS-61 and USGS-63, US Geological Survey; $\delta^{34}S$, USGS-42 and IAEA-S-2, International Atomic Energy Agency, Vienna, Austria) were used to check the accuracy of results. Analytical precision was < 0.10 ‰ for $\delta^{13}C$ and $\delta^{15}N$ values, and < 0.25 ‰ for $\delta^{34}S$ values.

2.5. GPS data processing and behavioural classification

A 'distance to colony' filter of 1 km radius was applied to GPS data of CS to remove colony locations and to account for social interactions and circling movements in the colony surroundings just before adults return to their nesting burrows at night [64]. GPS data collected from YLG and AG breeding on Berlenga and Deserta Islands were filtered manually to remove colony positions and avoid potential disturbance caused by inter-individual aggressions and landing. GPS data collected from YLG breeding in Porto were filtered at the nest-level once in these individuals it is usual the occurrence of feeding opportunities in the surrounding facilities. For all adults tracked, complete foraging trips were considered when the first and last positions were placed at the colony or nest.

Behavioural classification was carried out using the Expectation-Maximization binary Clustering (EMbC) algorithm [65], coupled with a post-processing smoothing to account for a potential mismatch when labelling positions [65,66]. EMbC bases on turning angle and instantaneous travel speed to discriminate four behavioural states: commuting-travelling (high velocity low turn, HL), resting or drifting on the water (low velocity low turn, LL), and extensive (high velocity high turn, HH) and intensive searching (low velocity high turn, LH) [65,67]. Foraging behaviour was considered when assigning positions as HH and LH. However, LL positions may also provide insights into foraging or feeding events in specific scenarios: i) in YLG, while on land, LL positions may indicate activities such as walking and searching for food during daylight [68]; ii) in gulls, repeated LL positions at the same spatial coordinates may suggest interactions with fishing vessels, *e.g.,* when fishermen are hauling their nets and birds are feeding on prey trapped at the surface; iii) in shearwaters, adults can adopt a 'sit-and-wait' feeding strategy while drifting on the water, particularly during daylight hours [69,70]. Therefore, LL positions were classified as foraging locations whenever these scenarios were observed in natural breeding colonies, respectively for gulls and shearwaters. By contrast, the classification of foraging locations in urban-dwelling YLGs is much more complex, once individuals can be "resting" at sightseeing spots (*e.g.,* lamps, buildings) just before the feeding attempts; additionally, urban dwellers may take advantage of the artificial light of the city to search for food at night. Thus, all positions within the urban environment classified as HH, LH, and LL were considered foraging locations.

2.6. *Classification of foraging habitat categories*

Foraging locations were extracted, and the type of habitat assigned based on the CORINE Land Cover dataset [71] in QGIS [72]. We considered four main 'habitat' types according to the habitat use of gulls: 'marine' (sea and open ocean), 'coastal' (beaches; coastal lagoons; estuaries; salt marshes; salines; intertidal flats; water bodies and courses), 'terrestrial' (agriculture fields; non-irrigated arable land; permanently irrigated land; pastures; natural vegetation and forests), and 'urban' (continuous and discontinuous urban fabric; industrial or commercial units; green urban areas; dump sites; fishing port areas; sport and leisure facilities; road and rail networks; construction sites; mineral extraction sites; airports). For CS, on the other hand, we opted to separate into two habitat categories: 'coastal marine' habitat, including all the foraging positions located within the continental shelf (200 m depth), and 'oceanic' habitat, including all the foraging positions located out of the continental shelf. The respective proportions of use of foraging habitat were calculated for each individual by dividing the number of foraging locations of a given category by the total of foraging locations.

2.7. *Data analysis*

Data visualisation, exploration, and statistical computations were carried out in R environment v4.3.1 [73].

2.7.1. *Relationship between THg and feeding habits inferred from stable isotopes*

We explored the relationships between blood THg concentrations and feeding ecology, inferred by stable isotope values. In a first analysis, we observed large differences in δ^{13}C, δ^{15}N, and δ^{34}S values among YLG colonies (see Table 1). Uneven spread of values within a given explanatory variable might result in the violation of homoscedasticity, essential for the correct fitting of a regression model [74]. Therefore, we opted to separate the analysis by colony: Deserta Island, Berlenga Island, and Porto. Secondly, we checked for multicollinearity among explanatory variables within each colony. Multicollinearity was checked using the variation inflation factor (VIF > 2.5, [75]) and Spearman correlation coefficients ($r_s > 0.50$) under the *usdm* R package [76]. δ^{15}N was identified as the most collinear variable when using YLGs breeding on Deserta Island and YLGs breeding on Porto (Spearman correlation, $0.56 < r_s < 0.88$, $p < 0.001$, VIF > 2.5). Therefore, we modelled δ^{15}N (trophic position model) separately from δ^{13}C and δ^{34}S (foraging habitat model), in a total of six univariate linear (LMs) or generalised linear models (GLMs) – three models using Hg $\sim \delta^{15}$N (one for each colony) and three models using Hg $\sim \delta^{13}\text{C} + \delta^{34}\text{S}$ (one for each colony, see Table S2). Specifically, a GLM fitted with gaussian family ('log' link) was used to test for the effect of blood δ^{15}N values on THg concentrations of YLGs breeding on Deserta Island, a GLM fitted with Gamma family ('log' link) for YLGs breeding on Berlenga Island, and a LM with log-transformed Hg for YLGs breeding in Porto (Table S2). For the foraging habitat models, two GLMs fitted with Gamma family ('log' link) were used to test the effect of blood δ^{13}C and δ^{34}S values on THg concentrations of YLGs breeding on Deserta and Berlenga Islands, while a LM with log-transformed Hg was used for YLGs breeding in Porto (Table S2). Two other GLMs were used to test the effect of feeding ecology (inferred from stable isotope values of δ^{13}C and δ^{15}N, and their interaction) on respective AG and CS blood THg concentrations (Table S2). Year was not included as a predictor since there were no annual differences in blood THg concentrations within each breeding colony (2021 *vs.* 2022: CS, YLG from Berlenga Island, and YLG from Porto, student's *t*-test, $0.43 < t < 1.50$, $p > 0.10$; 2021 *vs.* 2022 *vs.* 2023: AG and YLG from Deserta Island, one-way ANOVA, $0.05 < F < 0.76$, $p > 0.40$) and also because we considered two and three years of

Table 1

THg concentrations (μg g^{-1} dw) and carbon (δ^{13}C), nitrogen (δ^{15}N), and sulphur (δ^{34}S) isotope values (‰) measured in the blood of adult breeders: sample size (N), mean ± standard deviation (SD), and range values (minimum/maximum) are expressed for each sampling year. Superscript letters indicate significant differences computed using Kruskal-Wallis, followed by Dunn's test with Bonferroni adjustment ($\alpha < 0.05$).

	THg			δ^{13}C			δ^{15}N			δ^{34}S		
Species/Colony	N	Mean ± SD	Min/Max	N	Mean ± SD	Min/Max	N	Mean ± SD	Min/Max	N	Mean ± SD	Min/Max
Yellow-legged gull												
Porto	**29**	**0.98 ± 0.71^{a}**	**0.30/3.19**	**28**	**–20.7 ± 0.9^{a}**	**–23.1/–19.1**	**28**	**9.2 ± 1.0^{a}**	**8.1/11.9**	**28**	**11.0 ± 2.1^{a}**	**8.2/16.1**
2021	17	1.13 ± 0.81	0.30/3.19	17	–20.9 ± 1.0	–23.1/–19.1	17	9.3 ± 1.0	8.1/11.9	17	10.6 ± 2.0	8.2/14.9
2022	12	0.76 ± 0.48	0.31/1.99	11	–20.3 ± 0.7	–21.3/–19.4	11	9.0 ± 0.9	8.1/11.3	11	11.6 ± 2.1	8.5/16.1
Berlenga Island	**30**	**2.61 ± 1.01^{b}**	**1.03/5.73**	**27**	**–19.3 ± 0.5^{b}**	**–20.3/–18.5**	**27**	**13.0 ± 0.9^{c}**	**10.0/15.1**	**27**	**19.3 ± 0.9^{c}**	**15.5/19.9**
2021	16	2.63 ± 1.10	1.51/5.73	13	–19.4 ± 0.5	–20.1/–18.6	13	13.5 ± 0.7	12.5/15.1	13	19.5 ± 0.4	18.5/19.9
2022	14	2.58 ± 0.93	1.03/4.48	14	–19.3 ± 0.5	–20.3/–18.5	14	12.6 ± 0.9	10.0/13.6	14	19.1 ± 1.1	15.5/19.9
Deserta Island	**58**	**5.56 ± 2.67^{c}**	**0.50/12.62**	**57**	**–18.7 ± 1.0^{b}**	**–20.6/–15.9**	**57**	**11.9 ± 1.3^{b}**	**9.3/13.9**	**57**	**17.0 ± 2.3^{b}**	**8.4/19.5**
2021	17	5.56 ± 2.57	0.94/10.34	17	–18.8 ± 0.9	–20.6/–17.0	17	12.2 ± 0.9	9.6/13.6	17	17.5 ± 2.5	8.9/19.3
2022	16	5.56 ± 2.94	2.37/11.46	15	–18.6 ± 1.0	–19.8/–16.6	15	11.9 ± 1.3	9.7/13.9	15	17.4 ± 1.3	15.4/19.5
2023	25	5.55 ± 2.67	0.50/12.62	25	–18.7 ± 1.2	–20.4/–15.9	25	11.8 ± 1.4	9.3/13.9	25	16.4 ± 2.6	8.4/19.3
Audouin's gull	**49**	**7.16 ± 2.93^{c}**	**1.94/15.47**	**49**	**–19.4 ± 0.5^{b}**	**–20.1/–18.1**	**49**	**11.8 ± 0.6^{b}**	**10.9/13.4**			
2021	11	7.83 ± 2.99	4.42/13.88	11	–19.4 ± 0.7	–20.1/–18.1	11	12.4 ± 0.5	11.7/13.4			
2022	17	6.49 ± 2.55	3.42/11.44	17	–19.4 ± 0.4	–19.8/–18.6	17	11.7 ± 0.4	11.2/12.7			
2023	21	7.35 ± 3.22	1.94/15.47	21	–19.4 ± 0.5	–20.0/–18.4	21	11.7 ± 0.6	10.9/13.4			
Cory's shearwater	**30**	**2.01 ± 0.47^{ab}**	**1.26/3.41**	**26**	**–19.1 ± 0.4^{b}**	**–19.9/–18.4**	**26**	**11.9 ± 0.5^{b}**	**10.9/12.9**			
2021	15	2.08 ± 0.52	1.48/3.41	13	–19.2 ± 0.4	–19.9/–18.6	13	12.1 ± 0.5	11.2/12.9			
2022	15	1.94 ± 0.44	1.26/2.97	13	–19.1 ± 0.4	–19.6/–18.4	13	11.6 ± 0.4	10.9/12.3			

sampling as insufficient to assess any temporal trends in THg concentrations. Models were run using the 'lm' or 'glm' functions from *stats* and *lme4* R packages, respectively [73,77].

Isotopic niches were estimated to provide an overview of each population's feeding habits to integrate THg concentrations with the diet and foraging strategy of each population. The Bayesian estimation of standard ellipse areas (SEA_B) was calculated for the bivariate isotopic niches δ^{13}C - δ^{15}N (for all populations) and δ^{34}S - δ^{15}N (only for YLGs) to depict differences among isotopic niche sizes. Standard ellipse areas corrected for small sample sizes (SEA_C) including 40 % of data were used for visualisation purposes. All isotopic computations were carried out under several functions from the *SIBER* R package [78].

2.7.2. Relationship between THg and foraging habitats inferred from tracking data

We explored the relationships between blood Hg concentrations and the type of foraging habitat used, inferred by GPS foraging locations (Figs. S1 and S2). However, terrestrial habitats alone only included about 5.5 % (range: 0–24 %) and 1.8 % (range: 0–10 %) of foraging positions of YLGs breeding on Berlenga Island and Deserta Island, respectively, whereas urban habitats included on average 14 % (range: 0–50 %) and 18 % (range: 0–65 %) of foraging positions of YLG adults from the respective colonies. In an initial attempt to model blood THg concentrations *vs.* the proportional use of terrestrial and urban foraging habitats, we obtained a very low goodness of fit, reducing our trustfulness in those model outputs. Thus, we combined the proportions of 'terrestrial' and 'urban' habitat use (hereafter 'terrestrial') by YLGs, as the prey or food caught in these habitats exhibit more similar isotopic values than marine prey [79], which may indicate a more comparable exposure to Hg in terrestrial and urban habitats. By pooling terrestrial and urban habitats, we reduced the number of zeros of both categories from 19 to 6 individuals, which had affected the performance of the models computed for each habitat type. In the same line, for AGs, we grouped the foraging locations into "marine" and "non-marine" habitats (including the proportion of use of terrestrial, urban, and coastal habitats), since most of the foraging locations were within the marine environment (~95 %, range: 13–100 %; Fig. S1-D). Coastal habitats only represented 4 % (range: 0–14 %, but one individual showed 87 % of coastal habitat use) of AGs' foraging positions, whereas terrestrial and urban habitats represented less than 1 % (range: 0–5 %). Therefore, we coupled the marginal use of terrestrial and urban habitats with the use of coastal habitats (~5 % on average) as we considered that taking each category alone would bring noise to the model. Concerning CS, due to its strictly marine foraging strategy [24,64] – alternating between short foraging trips within the continental shelf and long foraging trips towards more deep oceanic areas (Fig. S2) – we separated marine foraging habitats into coastal marine habitats and oceanic habitats. Coastal marine habitats included GPS foraging locations within the continental shelf, while oceanic habitats included foraging locations outwards the continental shelf.

As stated for 'Hg ~ SI' relationships, models were computed separately for species, due to the disparate feeding habitats, diet, and foraging strategies (in a total of 3 models). Firstly, the proportions of use of foraging habitat (marine, coastal, and terrestrial) by YLGs were inspected for multicollinearity issues prior to modelling exercises, using the variation inflation factor (VIF > 2.5) [75] and spearman correlation coefficients ($r_s > 0.50$) under the *usdm* R package [76]. This inspection resulted in the exclusion of the proportion of marine habitat use from the analysis ($-0.81 < r_s < -0.73$, $p < 0.001$, VIF > 2.5). YLG breeding in Porto were not used in this modelling exercise due to the temporal mismatch between blood sampling and the tracking period, and thus, were only used for a general visual representation of the population. Secondly, we used GLMs adjusted to Gamma family distribution ('log' link function) to test the effect of the proportion of coastal and terrestrial habitat use (continuous explanatory variables) on blood Hg concentrations (continuous response variable). The colony was included as a categorical explanatory variable (Table S2) because the ecological context and location of YLGs' colony, *i.e.,* distance to food sources, fishery activities, and consequently resource availability have proven to drive different Hg concentrations on this species [53,54]. Thirdly, two other GLMs adjusted to Gamma family distribution ('log' link function) were used to test the effect of foraging on marine *vs.* non-marine and oceanic *vs.* coastal marine habitats on blood Hg concentrations of AG and CS adults, respectively (Table S2). The year was not included as an explanatory variable due to the similar annual Hg concentrations within each colony (Table 2). Models were run using the *lme4* R package [77].

2.7.3. Model selection and validation

Model selection was conducted using the Akaike's Information Criterion corrected for small sample sizes (AICc) to select the best model, *i.e.,* with the lowest ΔAICc, alongside with AICc weights and explained deviance used to infer about the effect of predictors on Hg concentrations see [80,81]. Whenever the null model was ranked as the best model, all predictors were considered as statistically non-significant. Models were checked for extreme values and validation was based on residuals normality and homoscedasticity [81] using the 'check_model' function from the performance R package [82]. Specifically, one individual was removed from the dataset of YLGs breeding on Deserta Island due to a very low δ^{13}C value (−23.1 ‰); two individuals were removed from the datasets of YLGs breeding in Porto and Berlenga Island due to a very high (16.1 ‰) and very low δ^{34}S values (15.5 ‰), respectively; two individuals were removed from the dataset of YLGs breeding on Berlenga Island due to extreme values on δ^{15}N values (10.0 ‰ and 15.1 ‰); three individuals were removed from the dataset of YLGs breeding in Porto due to very high δ^{15}N values (11.3 ‰, 11.5 ‰, and 11.9 ‰); and one AG was removed from the tracking dataset due to a very short tracking period (< 1 day). For detailed information on the initial sample sizes and those used for final model computations please consult Tables 1 and 2, respectively. Predicted values and confidence intervals were extracted from the best model using the 'ggpredict' function from *ggeffects* R package [83] and visualised using *ggplot2* R package [84].

3. Results

3.1. Blood THg concentrations in relation to feeding habits

Blood THg concentrations ranged from a mean of 0.98 ± 0.71 μg g^{-1} dw in YLG breeding in Porto to a mean of 7.2 ± 2.9 μg g^{-1} dw in AG breeding on Deserta Island (Table 1). Blood THg concentrations showed strong differences between the five breeding colonies (Kruskal-Wallis, χ^2 = 134.39, df = 4, $p < 0.001$). Post-hoc comparison test (Dunn's test) indicated that YLGs and AGs breeding on Deserta Island exhibited the highest blood Hg concentrations, followed by YLGs and CS breeding on Berlenga Island, and the lowest concentrations were found in YLGs breeding in Porto (Fig. 1, Table 1). Remarkably, 87 % of individuals were above 1.2 μg g^{-1} dw, the lowest threshold with reported Hg toxic effects in seabirds. Stable isotope values have only partially followed blood THg differences; specifically, δ^{13}C, δ^{15}N, and δ^{34}S values were the lowest in YLGs breeding in Porto, δ^{13}C values were the highest in YLGs breeding on Deserta Island, and δ^{15}N and δ^{34}S values were the highest in YLGs breeding on Berlenga Island (Table 1; Fig. 2A-B). The SEA_B showed no differences between CS and AG, which presented the narrowest δ^{13}C - δ^{15}N isotopic niches (Fig. 2C), while clear differences were observed among YLG colonies; specifically, those breeding in Porto and Deserta Island showed the widest δ^{13}C - δ^{15}N and δ^{34}S - δ^{15}N isotopic niches, while YLG breeding on Berlenga Island showed the narrowest niche space (Fig. 2D).

Model selection identified that δ^{15}N, δ^{13}C, and δ^{34}S as significant predictors of blood THg concentrations in YLGs from Deserta Island, while δ^{34}S alone explained blood THg concentrations in YLGs from Porto. No relationship was observed between THg and stable isotope values in YLGs from Berlenga Island (Table 2). In Deserta Island, δ^{15}N,

Table 2

Summary outputs including model specification, sample size (N), and Akaike's Information Criteria (AIC). The best ranked model outputs are specified, unless when the null model was ranked as best model. Significant effects are shown in bold, and the number of asterisks indicate the increasing level of significance ($\alpha < 0.05$, $\alpha < 0.01$, $\alpha < 0.001$). Abbreviations: *k*, number of parameters; AICc, Akaike's information criterion corrected for small sample sizes; ΔAICc, difference between AICc of the specific model and the best ranked model; w_i, AICc weights; Exp. Dev., explained deviance.

GLM Gamma family ('log' link)	*k*	AICc	ΔAICc	w_i	Exp. Dev.
Yellow-legged gull Deserta Island (N = 56)					
Full model: Blood THg ~ $\delta^{13}C + \delta^{34}S$					
Full model	3	223.00	0.00	1.00	0.59
$\delta^{13}C$	2	236.83	13.83	0.00	0.45
$\delta^{34}S$	2	247.07	24.08	0.00	0.35
Null model	2	269.64	46.64	0.00	0.00
	$\beta \pm$ SE	*t* value	p (> |*t*|)		
Intercept	5.45 ± 1.30	4.18	< 0.001	***	
$\delta^{13}C$	**0.30 ± 0.06**	**5.35**	**< 0.001**	***	
$\delta^{34}S$	**0.11 ± 0.02**	**4.56**	**< 0.001**	***	
GLM Gamma family ('log' link)					
Yellow-legged gull Berlenga Island (N = 26)					
Full model: Blood THg ~ $\delta^{13}C + \delta^{34}S$					
$\delta^{13}C$	2	72.38	0.00	0.33	0.11
Full model	3	72.84	0.46	0.26	0.18
Null model	2	72.85	0.47	0.26	0.00
$\delta^{34}S$	2	73.91	1.53	0.15	0.06
	$\beta \pm$ SE	*t* value	p (> |*t*|)		
Intercept	5.57 ± 2.97	1.88	0.07		
$\delta^{13}C$	0.24 ± 0.15	1.54	0.14		
LM Gaussian family					
Yellow-legged gull Porto (N = 27)					
Full model: log(Blood THg) ~ $\delta^{13}C + \delta^{34}S$					
$\delta^{34}S$	2	43.50	0.00	0.79	0.40
Full model	3	46.23	2.73	0.20	0.40
$\delta^{13}C$	2	53.46	9.96	0.01	0.13
Null model	2	54.69	11.19	0.00	0.00
	$\beta \pm$ SE	*t* value	p (> |*t*|)		
Intercept	−2.40 ± 0.54	−4.47	< 0.001	***	
$\delta^{34}S$	**0.19 ± 0.05**	**4.07**	**< 0.001**	***	
GLM Gamma family ('log' link)					
Audouin's gull (N = 48)					
Full model: Blood THg ~ $\delta^{13}C$ x $\delta^{15}N$					
$\delta^{15}N$	3	209.47	0.00	0.52	0.39
$\delta^{13}C + \delta^{15}N$	4	211.12	1.65	0.23	0.40
Full model	5	211.33	1.86	0.21	0.43
$\delta^{13}C$	3	214.36	4.89	0.04	0.33
Null model	2	231.32	21.85	0.00	0.00
	$\beta \pm$ SE	*t* value	p (> |*t*|)		
Intercept	−2.37 ± 0.87	−2.71	0.009	**	
$\delta^{15}N$	**0.37 ± 0.07**	**4.96**	**< 0.001**	***	
GLM Gamma family ('log' link)					
Cory's shearwater (N = 26)					
Full model: Blood THg ~ $\delta^{13}C$ x $\delta^{15}N$					
Null model	2	34.64	0.00	0.60	0.00
$\delta^{13}C$	3	37.08	2.44	0.18	0.01
$\delta^{15}N$	2	37.19	2.55	0.17	0.00
$\delta^{13}C + \delta^{15}N$	4	39.89	5.25	0.04	0.01
Full model	5	42.98	8.34	0.01	0.01

$\delta^{13}C$, and $\delta^{34}S$ values exhibited a positive relationship with blood THg in YLGs ($\delta^{15}N$: GLM, $\beta \pm$ SE, 0.32 ± 0.04, $t_{53} = 8.09$, $p < 0.001$; $\delta^{13}C$ and $\delta^{34}S$: GLM, slope $\delta^{13}C$, 0.30 ± 0.06; slope $\delta^{34}S$, 0.11 ± 0.02, $4.56 < t_{53} < 5.35$, $p < 0.001$; Fig. 3A-C, Table 2). In Porto, $\delta^{34}S$ showed a positive relationship with blood THg concentrations (Fig. 3D, Table 2). There was no relationship between $\delta^{15}N$ values and THg concentrations in the blood of YLGs from Porto (LM, 0.10 ± 0.20, $t_{23} = 0.53$, $p = 0.60$) or Berlenga Island (GLM, 0.20 ± 0.12, $t_{23} = 1.60$, $p = 0.12$). AGs showed a positive relationship between Hg and $\delta^{15}N$ values (0.37 ± 0.07, $t_{44} = 4.96$, $p < 0.001$; Table 2, Fig. 4), while no relationship was found for $\delta^{13}C$ values or its interaction with $\delta^{15}N$ values (Table 2). There was no relationship between $\delta^{13}C$ or $\delta^{15}N$ values, nor their interaction with Hg concentrations in CS (Table 2).

3.2. *Blood THg concentrations in relation to foraging habitats*

YLGs exhibited different foraging strategies among colonies (Fig. 5A-C): YLG breeding on Berlenga Island foraged extensively on marine habitats (~ 78 %; Fig. 5A) while those breeding on Deserta Island foraged preferentially on marine habitats (~51 %), although coastal and terrestrial/urban habitats were also important for this population (~26 % and 23 %, respectively; Fig. 5B). Finally, YLG breeding in Porto (urban colony) exhibited a clear preference for foraging on urban habitats (~59 %) and less preference for marine and coastal habitats (Fig. 5C). Concerning AG and CS, both species exhibited a marine-based foraging strategy (Fig. 5D-E); CS foraged more on coastal marine (~60 %) rather than on oceanic habitats (~40 %; Fig. 5E).

Model selection indicated that the colony and the proportion of use of terrestrial habitats were significant predictors of blood THg concentrations in YLGs. (Fig. 6). The model showed a moderate negative relationship between THg concentrations and the proportional use of terrestrial habitats ($\beta \pm$ SE: slope -0.71 ± 0.32, $t_{36} = -2.21$, $p = 0.03$, AICc = 143.93; Fig. 6), as well as higher blood THg concentrations in YLGs from Deserta Island compared to those from Berlenga Island ($p < 0.001$). Conversely, the proportional use of marine foraging habitats by AG and of oceanic habitats by CS were not significant predictors of THg variations in blood (AG, $\beta \pm$ SE: slope 0.68 ± 2.39, $t_{16} = 0.28$, $p = 0.78$; CS, $\beta \pm$ SE: slope -0.15 ± 0.19, $t_{21} = -0.80$, $p = 0.43$).

4. Discussion

This study is the first to relate THg concentrations, trophic ecology, and foraging habitats of gull and shearwater species breeding along the western and southern coasts of Portugal. We observed large differences in blood THg concentrations between colonies, but not between species breeding at the same location. In gulls from Deserta Island, higher trophic positions (inferred by higher $\delta^{15}N$ values) were associated with increased Hg contamination, a pattern not observed in shearwaters or in YLGs from Berlenga Island or Porto. A more terrestrial foraging strategy in YLGs (inferred by lower $\delta^{13}C$ and $\delta^{34}S$ values and disclosed by

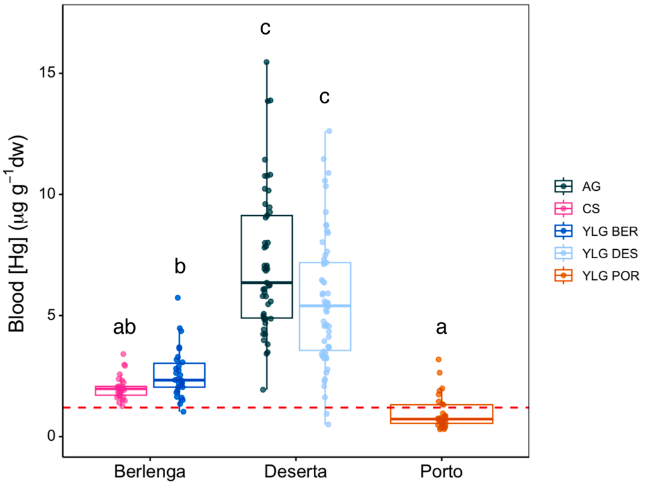

Fig. 1. Jitter plots of blood THg concentrations (µg g^{-1} dw) in yellow-legged gulls (YLGs), Audouin's gulls (AGs) and Cory's shearwaters (CSs) breeding on five breeding colonies located along the western and southern coasts of Portugal. Boxes display the range between 25 % and 75 % quantiles, error bars extend to a maximum (97.5 %) and minimum values (2.5 %), the solid black line represents the mean, and points represent the raw data. Letters indicate significant differences computed using Dunn's test with Bonferroni adjustment. The red dashed line represents blood Hg concentrations from which ecophysiological impacts were already observed in birds [11]. Abbreviations: AG, Audouin's gull; CS, Cory's shearwater; YLG: yellow-legged gull; BER, Berlenga Island; DES, Deserta Island; POR: Porto.

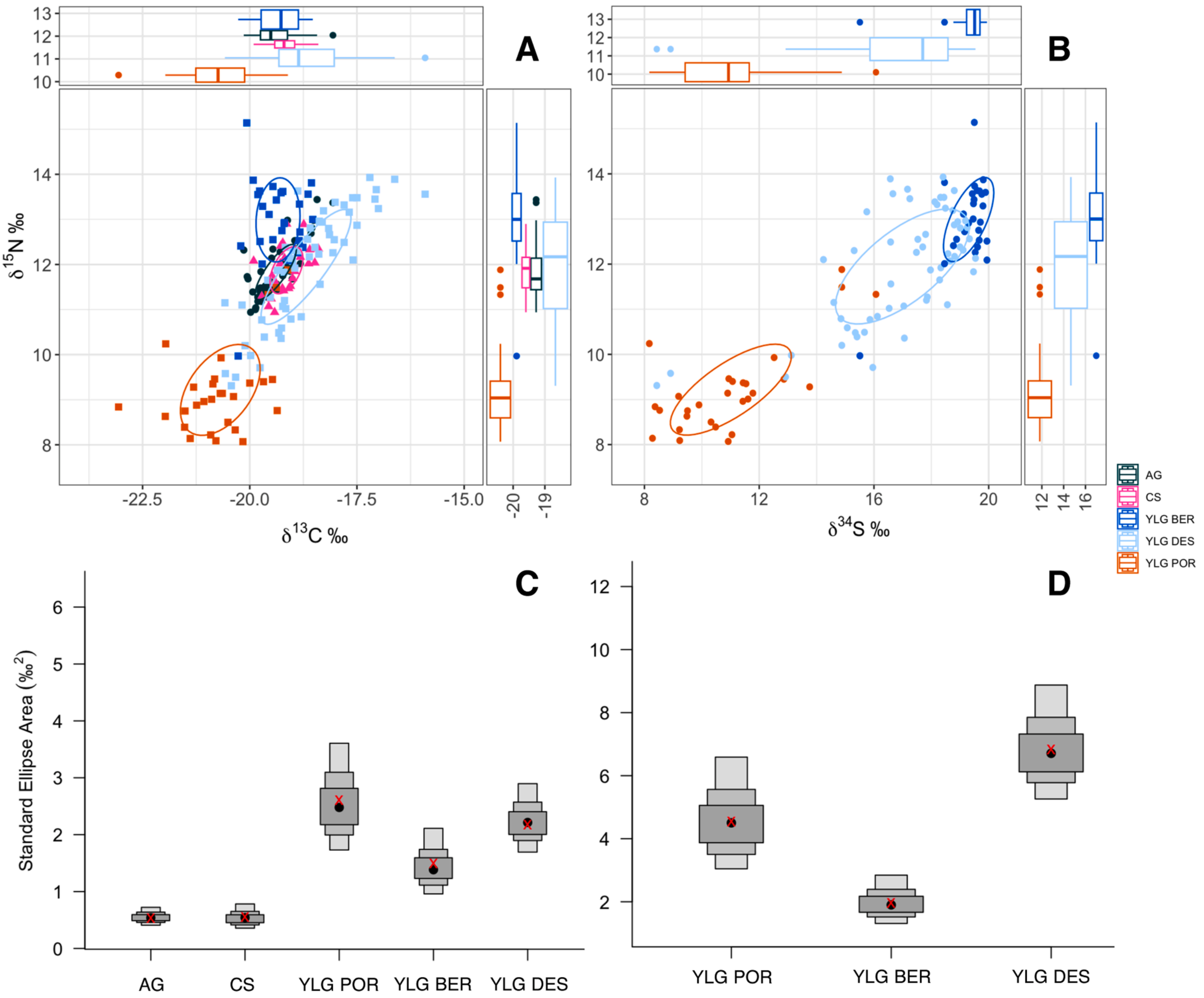

Fig. 2. Isotopic niche space represented by standard ellipse areas computed using A) δ^{13}C - δ^{15}N (‰) measured in the blood of gulls and shearwaters breeding on the city of Porto, Berlenga and Deserta Islands, and B) δ^{34}S - δ^{15}N (‰) measured in the blood of yellow-legged gulls (YLGs) breeding on the three study locations. Ellipses were computed using 40 % of total data and represent the standard ellipse area corrected for small sample sizes (SEA$_C$). Boxplots show the median δ^{13}C, δ^{15}N, and δ^{34}S values, the first (25 %) and third (75 %) quartiles, the lowest and highest values of the 95 % confidence intervals, CI (solid lines), and outliers (dots). C-D) SEA (‰^{2}), Bayesian SEA (SEA$_B$) represented by the black dot, and SEA$_C$ represented by the red cross of the respective A and B biplots. Abbreviations: AG, Audouin's gull; CS, Cory's shearwater; YLG: yellow-legged gull; BER, Berlenga Island; DES, Deserta Island; POR: Porto.

foraging positions) resulted in lower blood THg concentrations. These findings highlight the critical role of dietary exposure (*via* trophic transfer) in driving Hg contamination of seabirds [12,13,16–18]. However, the lack of consistency in the relationships between stable isotope values and Hg concentrations between species and between YLG populations suggest potential influences of differing background Hg contamination or the effect of unassessed factors such as age, sex, physiology, or phylogeny.

4.1. THg concentrations, toxicity, and potential health risks

Blood Hg contamination has been widely studied in seabirds [reviewed in 1]. Due to the relatively short half-life of Hg in blood (up to 2 months; [44]), blood samples are valuable for assessing recent exposure to Hg, particularly during the breeding period *e.g.,* [16, 58, 59, 85]. Additionally, more than 90 % of THg in bird blood is in the form of MeHg [86], so THg concentrations can be a reliable proxy of MeHg concentrations and be used to assess their toxicological risks [9,10,87].

In our study, blood THg concentrations ranged from relatively lower in urban-dwelling YLGs (0.98 ± 0.71 μg g^{-1} dw) and in CS and YLGs breeding on Berlenga Island (YLG, 2.6 ± 1.0 μg g^{-1} dw; CS, 2.0 ± 0.5 μg g^{-1} dw) to high in YLGs and AGs breeding on Deserta Island (AG, 7.2 ± 2.9 μg g^{-1} dw; YLG, 5.6 ± 2.7 μg g^{-1} dw). These findings suggest that gulls breeding on Deserta Island may be more vulnerable to the toxic effects of Hg, while YLGs breeding in Porto appear to be at lower risk. Indeed, based on Hg toxicity benchmarks for blood in birds see [11], 11 % of the individuals were classified as being at no risk (< 0.95 μg g^{-1} dw, N = 22, converted from 0.2 μg g^{-1} wet weight assuming a blood moisture content of 79 %, [88]), 52 % were categorised as at low risk (0.95–4.8 μg g^{-1} dw, N = 101), 37 % as at moderate risk (4.8–14.3 μg g^{-1} dw, N = 72), and one single individual AG was classified as at high risk (14.3–19.0 μg g^{-1} dw). Notably, nearly 70 % of AG and YLG sampled on Deserta Island (N = 72) exceeded the threshold for moderate risk of Hg contamination, raising ecotoxicological concerns for these populations. Similar blood THg concentrations have been reported in YLG breeding on the Île de Ré on the west coast of

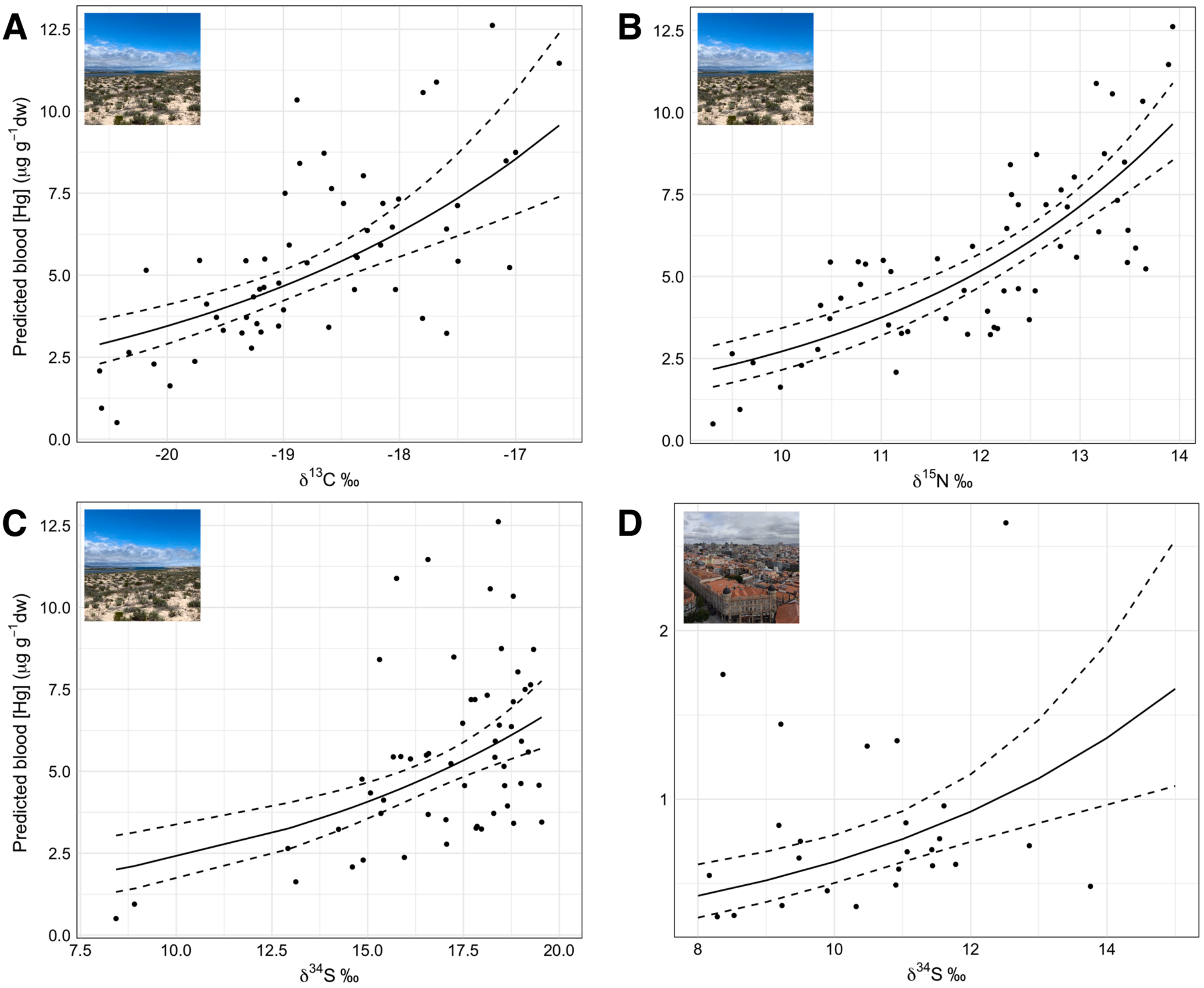

Fig. 3. Predicted blood THg concentrations (μg g^{-1} dw) increased with increasing A) δ^{13}C, B) δ^{15}N, and C) δ^{34}S values in adult yellow-legged gulls (YLGs) breeding on Deserta Island and increased with increasing D) δ^{34}S values in adult YLGs breeding in Porto (see Table 2). Solid lines represent the predicted lines, dashed lines represent the 95 % CI, and the points represent the blood THg concentrations predicted by the best selected model.

France (mean ± SD, 6.0 ± 2.7 μg g^{-1} dw [16], and in Dragonera, Balearic Islands (5.9 ± 3.7 μg g^{-1} dw) [54]. In seabirds, such blood Hg concentrations are known to affect physiology, behaviour, and reproduction reviewed in [87], through endocrine disruption [89–91], and may reduce breeding success, fitness, and recruitment *e.g.,* [92–97]. However, other factors not assessed here, like selenium (Se) both in terms of concentrations and physiochemical forms, can mitigate Hg toxicity [98,99]. Selenium is an essential trace element recognised for its capacity to reduce Hg toxicity by forming a biologically inert and insoluble complex, mercury selenide [99,100]. In a recent study carried out on the same breeding colonies, adults exhibited a blood Se:Hg molar ratio above 4 [53], outlined as the threshold of Se–Hg needed to effectively demethylate MeHg [100]. This suggested that the quantity of blood Se could be high enough to allow an effective protection against Hg toxicity in these populations. Even so, in the present study, 87 % of individuals displayed blood THg concentrations above 1.2 μg g^{-1} dw, threshold concentration in blood cells associated with detectable physiological impacts in seabirds [11,91]. This underlines the need for further research to investigate whether these elevated blood THg concentrations have negative effects on these populations and to understand the potential long-term consequences for reproduction and demography.

In Porto, nearly 70 % of the YLG sampled (N = 20) were classified as being at no risk, as reported for California gulls (*L. californicus*), for herring gulls (*L. argentatus*) from the Baltic Sea and from the Northeastern U.S. (range of mean blood Hg: 0.5–1.4 μg g^{-1} dw) [17,22,101], although much lower than urban nesting black-legged kittiwakes (*Rissa tridactyla*) from Boulogne-sur-Mer, Northern France (range of mean blood Hg: 2.7–6.9 μg g^{-1} dw) [102]. With the exception of one individual, adults breeding on Berlenga Island (*i.e.,* both YLG and CS) were classified as at low risk from Hg contamination, similar to those reported for lesser black-backed gulls (*L. fuscus*) in Northeast Atlantic, western gulls (*L. occidentalis*) in Northeast Pacific, and YLG in the western Mediterranean Sea (range of mean blood Hg: 2.0–3.8 μg g^{-1} dw) [16,21,54] and medium-sized Procellariiformes (range of mean blood Hg: 0.3–3.3 μg g^{-1} dw) [13,23,103–105]. These findings suggest an overall negligible risk of Hg toxicity for YLGs from Porto and for CS and YLGs from Berlenga Island [11,87].

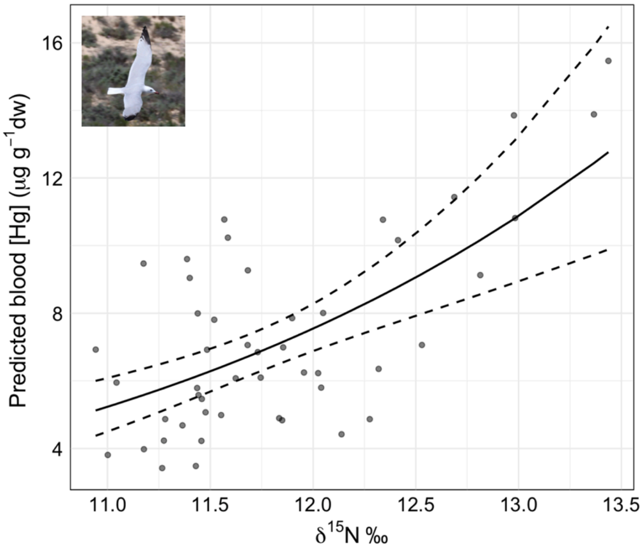

Fig. 4. Predicted blood THg concentrations (µg g^{-1} dw) increased with increasing δ^{15}N values ($\beta \pm$ SE: slope 0.37 ± 0.07, intercept −2.37 ± 0.87, N = 48, ΔAICc$_{\text{Null model}}$ = 21.85, see Table 2) in Audouin's gulls (AGs) breeding on Deserta Island. Solid line represents the predicted line, dashed lines represent the 95 % CI, and the points represent the blood THg concentrations predicted by the best selected model. Photo credits M.I. Laranjeiro.

4.2. THg concentrations in relation to seabird feeding and foraging strategies

The positive relationship between blood THg concentrations and stable isotope values of δ^{13}C, δ^{15}N, and δ^{34}S in YLGs from Deserta Island is consistent with findings from previous studies on gulls *e.g.,* [16, 17, 54]. Higher blood THg concentrations were observed in YLGs feeding at a higher trophic position (inferred by higher δ^{15}N values) and on marine prey (inferred by higher δ^{13}C and δ^{34}S values) rather than terrestrial items. Similarly, AGs feeding at a higher trophic position exhibited higher blood THg concentrations, despite the absence of a clear relationship with their feeding habitats. This pattern aligns with higher Hg biomagnification rates in marine *vs.* terrestrial food webs, as well as the higher MeHg production typically found in marine ecosystems [5,6]. The higher complexity and length of marine food webs allows Hg to biomagnify more efficiently, reaching much higher concentrations in marine biota compared to their terrestrial counterparts [7,106]. In fact, THg concentrations observed between the gull populations are consistent with the composition of the diet at each breeding site. Pellet analysis shows that YLGs from Berlenga Island primarily rely on the pelagic Henslow's swimming crab (~ 75 %), and secondly on pelagic and demersal fish (~ 15 %) [56,107]; YLGs from Deserta Island predominantly feed on pelagic and demersal fish (~ 35 % and 25 % of the diet, respectively) [56]; YLGs from Porto feed on higher amounts of anthropogenic-derived resources such as urban waste and refuse items [57]; and AGs feed mostly on pelagic and mesopelagic and demersal fish (~ 60 % and 25 % of the diet, respectively) [56]. By contrast, CS feed mainly on pelagic fish and cephalopods [24,55]. Demersal and mesopelagic fish are identified to be potentially significant sources of Hg contamination [12,48], whereas crustaceans, pelagic fish, cephalopods, and anthropogenic-derived resources appear to contribute to a lesser extent to Hg contamination. In fact, demersal and mesopelagic species usually occupy higher trophic positions and are more enriched in Hg compared to epipelagic fish, crustaceans, and cephalopods [12, 108–110], as in low-oxygen waters, increased bacterial activity promotes the formation and bioaccumulation of MeHg, which ultimately increases the baseline level of MeHg [6,109,111]. Therefore, the intense fishery-associated foraging behaviour in gulls from Deserta Island [56, 62] – particularly to trawlers that operate extensively in the area [112] – likely explains the higher concentrations of THg observed in the blood of adults and may present a high risk for these populations. In contrast, the greater reliance on terrestrial and/or anthropogenic resources of lower trophic levels appears to have contributed to the lower Hg contamination of the YLGs from Porto [16,17]. Therefore, these results highlight the critical role of dietary choices in influencing Hg exposure and intake in these populations.

Interestingly, a strong relationship between blood stable isotope values and THg concentrations was observed only in gulls from Deserta Island, with no such relationship detected in the other gull populations or in shearwaters. This suggests that the relationship between isotopic composition and Hg concentrations should be examined at both species and colony level [113]. Variations in the baseline concentration of Hg in different food webs can influence the exposure of top predators, which can lead to weak or incorrect relationships if regional differences are not considered [113]. In this study, higher δ^{13}C, δ^{15}N, or δ^{34}S values in YLGs from Berlenga Island have not predicted blood THg concentrations. Moreover, the highest δ^{15}N and δ^{34}S values detected in this YLG population could have suggested *a priori* higher THg concentrations, which was not observed. This rather suggests that YLGs may not only be exposed to site-specific background Hg contamination but may also experience variation in baseline nitrogen isotopic composition [113], as well as variation in sulphur isotopic composition due to highly different sulphur inputs from land [114–116]. In more detail, it is possible that: 1) the baseline concentration of Hg in the food web at Berlenga Island, along with its bioaccumulation factor, could be lower, resulting in less contaminated prey and, consequently, lower Hg concentrations in gulls' blood; 2) the prey consumed by YLGs at Berlenga and Deserta Islands may occupy distinct trophic positions, but with a shifted isotopic baseline (as suggested by the lower δ^{15}N values). Additionally, it is rational that YLGs from Berlenga Island exhibit higher δ^{34}S values, given that the adults mainly rely on a highly sulfate-enriched environment to search for their prey, *i.e.,* the marine environment [17,49]. In contrast, YLGs from Deserta Island are subjected to a broader array of sulphur sources (*i.e.,* sulphates and sulphides) from freshwater, brackish water, and seawater systems, as the breeding colony is located within a saltmarsh ecosystem, *i.e.,* Ria Formosa Natural Park. Freshwater and saltmarshes are simultaneously depleted in sulphate and enriched in sulphide anions compared to the marine environment [114–116], which explains the intermediate δ^{34}S values observed in consumers from coastal systems compared to freshwater (range: –10–10 ‰) and marine consumers (range: 15–21 ‰) [31,117–119]. However, studies carried out in consumers in coastal systems such as estuaries have identified that a very small amount of sulphate-rich seawater added to freshwater is enough to mask freshwater's δ^{34}S signature [120], which may also explain the high δ^{34}S values of YLGs from Deserta Island (mean ± SD: 17.0 ± 2.3 ‰; Table 1). Moreover, the sulphur isotope does not exhibit a noticeable increase from one trophic level to the next [121,122], making perceptible the influence of the saltmarsh system on δ^{34}S values even on top predators like gulls. Thus, it is plausible that the largely different ecological contexts of YLG's breeding colonies: Berlenga, a neritic island; Deserta, a sandy island within a saltmarsh ecosystem; and Porto, an urban centre crossed by a major river, are influencing baseline δ^{34}S values.

The pronounced mixed-foraging strategy of YLGs from Deserta Island possibly explains the strong relationship observed between blood stable isotope values and THg concentrations. This supported the characterisation of this population as generalist, although composed of specialised individuals, a pattern commonly observed in other gull species [16,17, 21,22] and corroborated by spatial data. In contrast, YLGs from Berlenga Island appear to be more specialised at the population level, as evidenced by the narrower isotopic niche and less variable spatial distribution (Fig. 2 and S1). This population predominantly relies on marine habitats for foraging, targeting prey such as the superabundant Henslow's swimming crab (naturally caught [123]), and pelagic or demersal

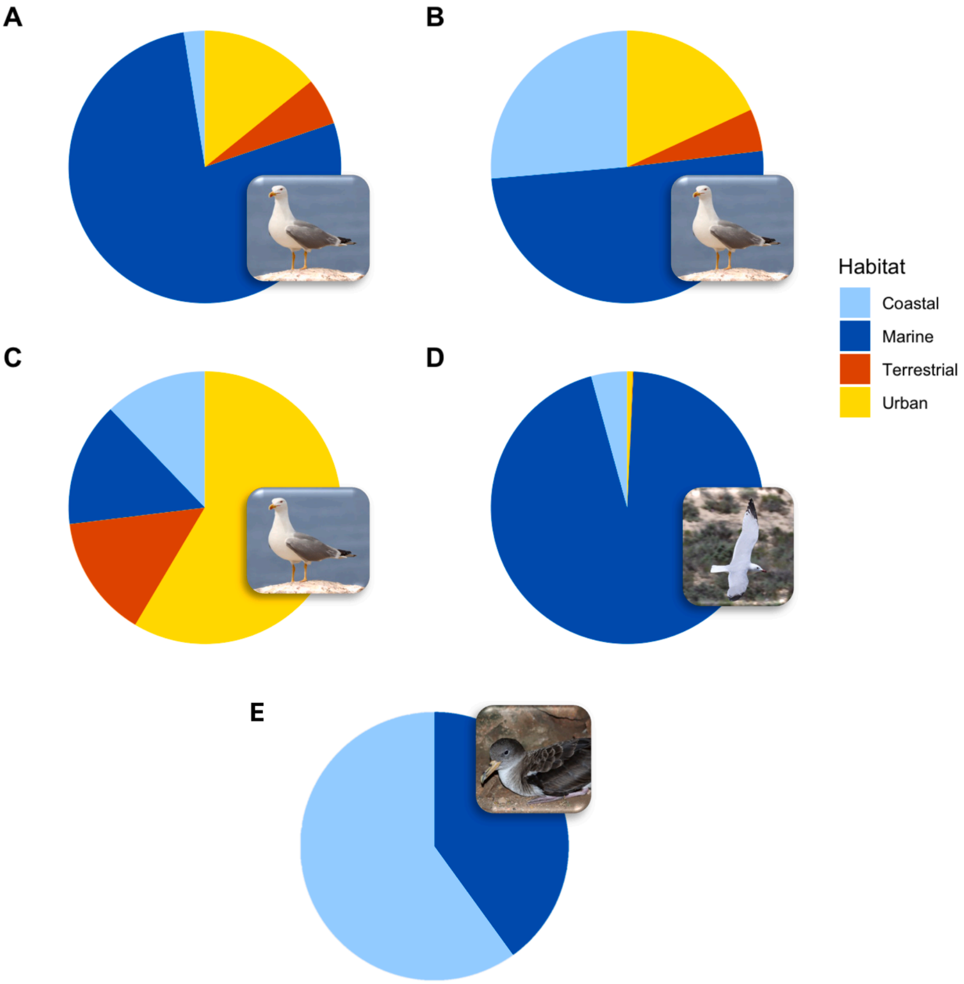

Fig. 5. Pie charts representing the proportion of type of habitat used while foraging by yellow-legged gulls (YLGs) breeding on (A) Berlenga Island, (B) Deserta Island, (C) Porto, (D) Audouin's gulls (AGs) breeding on Deserta Island, and (E) Cory's shearwaters (CS) breeding on Berlenga Island. Foraging locations were labelled through the classification GPS positions by the EMbC algorithm [65]. Habitat types were classified according to CORINE Land Cover dataset [71] and the procedure is described in more detail in "Classification of foraging habitat categories" section in the Materials and Methods. Photo credits M.I. Laranjeiro.

fish obtained in association with purse seine and trawler vessels, respectively [27,56,107]. Thus, the higher foraging and feeding consistency within the YLG population breeding on Berlenga Island may have hindered the detection of a causal relationship between foraging behaviour and THg concentrations. However, to ascertain an isotopic baseline shift between the food webs of Deserta and Berlenga Islands, the accurate estimation of gulls' trophic position becomes crucial. Compound-specific nitrogen stable isotope analysis of amino acids [124,125] has proven effective in estimating trophic positions in crustaceans [126], fish [127], and seabirds [128–130], offering a promising approach to address this gap. Future research should prioritise accurate estimation of site-specific trophic positions to determine whether observed differences in Hg contamination are attributable to site-specific background Hg contamination and bioaccumulation factors, or to variations in trophic positions of adults.

Unexpectedly, we found no differences in blood THg concentrations between CS and YLGs from Berlenga Island, despite the large dissimilarities in their isotopic composition and known dietary differences [24,56]. However, during the sampling years 2021 and 2022, some of the highest blood $\delta^{15}N$ values for YLGs from Berlenga Island were recorded, alongside with some of the narrowest isotopic niches [131]. This suggests a more specialised diet during these years, composed of higher trophic level prey, when compared to previous years. Indeed, Ceia et al., [131] found a positive relationship between blood $\delta^{15}N$ values and fisheries' landings in YLGs from Berlenga Island, particularly of demersal carnivorous – two-banded seabream (*Diplodus* spp.) and the European hake (*Merluccius merluccius*) – and pelagic mid-trophic species – European pilchard (*Sardina pilchardus*) and several mackerel species (*Scomber* spp. and *Trachurus* spp.). These species are likely to exhibit higher $\delta^{15}N$ values (range: 10–12 ‰; [27,132,133]) compared to the super abundant Henslow's swimming crab (range: 5.8–6.2 ‰; [27,134]), which could explain the increased $\delta^{15}N$ values observed in YLGs during the study period. Additionally, Henslow's swimming crab have shown enriched sulphur isotopic composition compared to pelagic fish in Galicia and Asturias, Spain (Henslow's swimming crab, range: 21.5–23 ‰; pelagic fish, range:19.5–21 ‰) [79], while pelagic fish exhibited overall higher $\delta^{34}S$ values than demersal fish (pelagic fish, range: 15.5–17.5 ‰; demersal fish, range: 11.5–17 ‰), despite the high variability among species [135]. It is therefore reasonable to suppose that blood THg concentrations of YLGs have reached values similar to those observed in shearwaters over these years, as a result of increased fish consumption. Nevertheless, feeding and foraging habits are not the sole drivers of Hg exposure in seabirds.

Other factors, such as sex and age, as well as broader traits like morphological, physiological, and phylogenetic differences, may significantly contribute to Hg variation [3,136–139]. For instance, in birds, females often exhibit lower Hg burdens than males, due to their capacity to depurate Hg through egg-laying [140,141], while within-individual Hg accumulation has been shown no clear pattern with age [89,90,136,137,142,143], and other has even evidenced

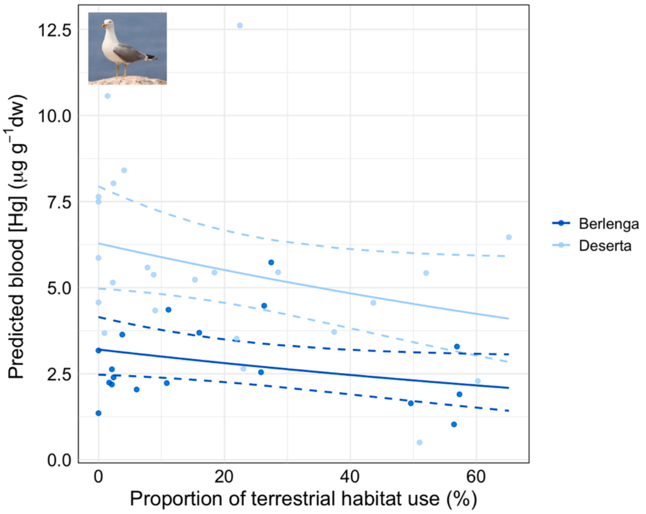

Fig. 6. Predicted blood THg concentrations (μg g^{-1} dw) decreased with increasing proportion of terrestrial habitat use (%) ($\beta \pm$ SE: slope -0.71 ± 0.32, intercept: 1.18 ± 0.11, $p = 0.03$, $\Delta \text{AICc}_{\text{Null model}} = 21.43$, N = 39) by YLGs breeding on Berlenga Island (blue) and Deserta Island (light blue). Solid lines represent the predicted lines, dashed lines represent the 95 % CI, and the points represent the blood THg concentrations predicted by the best selected model. Photo credits M.I. Laranjeiro.

sex-related capacity to demethylate MeHg [144]. Additionally, phylogenetic differences also account for substantial variation in THg concentrations, as observed in albatrosses [3,139]. Body size differences between larger and smaller *Thalassarche* spp. seemed to explain variability in feather Hg concentrations [3]. Furthermore, Borgå et al., [138] reported species-specific differences in essential and non-essential elements, despite regional similarities, highlighting the role of physiological requirements and phylogenetic regulation capacity in shaping element concentrations among seabirds. These traits likely impact exposure, accumulation, and detoxification pathways, reflecting both ecological roles and evolutionary constraints that shape Hg dynamics in seabirds.

Strikingly, the lowest blood THg concentrations and stable isotope values were found in YLGs breeding in Porto. Indeed, lower THg concentrations was anticipated in this urban population, as these gulls primarily rely on urban wastes and on nearby landfills [57,61], generally feeding on lower trophic position prey and/or food with lower Hg concentrations than marine prey [79,108–110]. Despite the broad isotopic niches observed for urban YLGs (Fig. 2) – which might have facilitated the detection of an association between blood stable isotope values and THg concentrations – only sulphur isotopic composition showed a positive association with THg concentrations. This suggests that individuals foraging more in sulphur-enriched habitats, such as marine environments [31,129], exhibited higher blood Hg concentrations. Sulphur isotopes are particularly effective for distinguishing reliance on marine *vs.* terrestrial food webs in birds [16,17,34], making δ^{34}S a valuable tool for assessing gulls' trophic niches [31,79,145]. Conversely, we found no relationship between blood δ^{13}C or δ^{15}N values and THg concentrations in urban YLGs or in shearwaters. The lack of a relationship for shearwaters could be attributed to the narrow isotopic niche during the breeding period, potentially due to the higher degree of dietary specialisation of this population. Despite their wide foraging ranges [24,64], CS adults breeding on Berlenga Island primarily exploit similar foraging habitats, mostly within the Portuguese continental shelf (< 200 m depth), a region known for its coastal upwelling during the summer season [146,147]. Such foraging consistency likely leads to a less diversity of prey caught, narrowing the isotopic niche and resulting in more uniform exposure to Hg across adults, which could make it more challenging to detect a relationship between Hg concentrations and stable isotope values *e.g.,* [54,105]. Urban YLGs, on the other hand, because of the wide variety of food sources explored, their high predictability, but unpredictable distribution [148], may have buffered carbon and nitrogen isotopic composition, *i.e.,* higher isotopic variability [149,150], challenging the detection of a pattern with Hg concentrations. Therefore, we further stress the need to include δ^{34}S as a predictor of Hg in studies of this nature, as it accounts for variation in Hg among individuals within the same population, providing more precise relationships between resource use in mixed-foraging species and Hg uptake [16,17].

The isotope composition was consistent with the proportional use of foraging habitats by adults, although this relationship was observed only in YLGs. In particular, higher δ^{34}S values were observed in YLGs from Berlenga Island which mostly exhibited a marine foraging strategy, while those from Deserta Island exhibited lower δ^{34}S values and a higher use of terrestrial habitats. Nonetheless, the use of terrestrial habitats was associated with lower blood THg concentrations both in YLG from Berlenga and Deserta Islands. This result aligns with previous telemetry studies on other coastal gulls species such as herring, lesser black-backed, and great black-backed (*L. marinus*) gulls in the Northeast Atlantic [16], herring gulls from the Northeastern U.S. [22], and western gulls in the Northeast Pacific [21]. Furthermore, this underlines the influence of the "marine-terrestrial" gradient to explain variations in the exposure to Hg [16,17,151]. Conversely, the uniform use of marine foraging domains by AGs and CSs limited the ability to detect a relationship between habitat use and blood THg concentrations. It is important to note that the tracking period did not align with the timeframe reflected by blood Hg concentrations, as blood samples were collected during GPS deployment sessions. This mismatch may have some impact on the observed relationships between Hg concentrations and the use of foraging habitat, although previous studies do not suggest significant changes in foraging locations during the incubation period [but see 43 and references therein]. While a moderate negative relationship was observed between the proportional use of terrestrial habitats and blood THg concentrations in YLGs, this might be attributed to the high degree of individual specialisation (particularly on Deserta Island) and the repetitive use of specific foraging habitats, which might stabilise blood Hg concentrations over time.

5. Conclusion

This study highlights the critical role of seabird foraging strategies in influencing Hg uptake. Blood THg concentrations were strongly associated with trophic position in gulls, reinforcing the value of δ^{15}N as a reliable indicator of Hg contamination and biomagnification. Feeding habitats also influenced Hg variation, but this effect was significant only in YLG, likely due to the high individual variability in foraging strategies both within and among breeding colonies. These findings underline the potential of these species as bioindicators of environmental contamination during the breeding season. We advocate for further studies integrating stable isotopes and telemetry methods to better identify the sources of Hg and other contaminants in seabirds.

In Deserta Island, gulls exhibited high THg concentrations compared to other colonies and to shearwaters, raising ecotoxicological concerns. Remarkably, 87 % of the sampled adults exceeded the lowest Hg toxicity benchmark for impaired reproduction in seabirds, *i.e.,* 1.2 μg g^{-1} dw, suggesting potential deleterious effects [89–91]. This included 100 % of AG and CS adults sampled at Deserta and Berlenga Islands, respectively, which could be critical as AG is currently classified as Vulnerable (VU) according to the IUCN Red List [152], and the breeding population of CS from Berlenga archipelago appears to be declining since 2015 [153]. Importantly, future studies should also measure blood Se, as this essential element is involved in the detoxification of Hg, thereby mitigating some of its toxicity, while further research is needed to explore physiological, health, and reproductive risks associated with these high Hg concentrations in the short to medium term. Additionally, the high

THg concentrations observed in adult birds warrants a comprehensive risk assessment of Hg exposure in chicks from the same colonies.

Although urban gulls showed limited exposure to Hg, they are likely exposed to other pollutants common in urban environments, such as lead (Pb), plastics, pesticides, and industrial by-products [53,154–156]. Concurrently, gulls from Deserta Island have been affected by botulism, which could exacerbate inflammatory processes and increase their susceptibility to parasite infections, potentially disrupting their physiology and overall health condition [157]. These additional factors raise significant concerns about the combined effects of multiple contaminants and their cumulative effects on the health of gulls inhabiting in both urban and natural colonies.

Environmental implication

This study addresses how foraging strategies influence Hg contamination in seabirds along the Portuguese coast. Dietary tracers and foraging locations reveal that foraging behaviour predicts blood Hg concentrations, with regional variations emphasising the need for species- and colony-specific assessments. While overall risk of Hg toxicity seems low, 87 % of adults exceeded reproductive impairment thresholds in seabirds. However, recent data on selenium (Se) in blood suggest that this essential element is present in sufficient concentrations to protect these populations against Hg toxicity. Ongoing monitoring is crucial to address Hg pollution, mitigate potential impacts on seabird populations, and inform conservation strategies.

CRediT authorship contribution statement

dos Santos Ivo: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Ramos Jaime A.:** Writing – original draft, Supervision, Resources, Investigation, Funding acquisition, Conceptualization. **Ceia Filipe R.:** Writing – review & editing, Methodology. **Pais de Faria Joana:** Writing – review & editing, Methodology. **Pereira Jorge M.:** Writing – review & editing, Methodology. **Seco José:** Writing – review & editing, Methodology. **Cerveira Lara R.:** Writing – review & editing, Methodology. **Laranjeiro Maria I.:** Writing – review & editing, Methodology. **Brault-Favrou Maud:** Writing – review & editing, Methodology. **Veríssimo Sara N.:** Writing – review & editing, Methodology. **Bustamante Paco:** Writing – original draft, Supervision, Resources, Investigation, Funding acquisition, Conceptualization. **Paiva Vitor H.:** Writing – original draft, Supervision, Resources, 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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2025.137673.

Data availability

Data will be made available on request.

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