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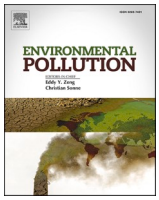

Spatial variation of mercury contamination in yellow-legged gulls (*Larus michahellis*) in the Western Mediterranean☆

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A B S T R A C T

Mercury (Hg) is a global pollutant of major concern in marine and coastal environments. In the Mediterranean Sea, Hg concentrations in biota are higher than in other seas, even when seawater concentrations are similar. Seabirds, as marine top predators, can reflect Hg contamination on a large spatial scale. By sampling seabirds at 17 different breeding colonies, we evaluated Hg concentrations of yellow-legged gulls (*Larus michahellis*) in the occidental Mediterranean basin in 2021 and 2022. More specifically, we investigated spatial variation of Hg contamination in both chicks and adults as well as associated toxicological risks through the use of blood and feathers, which reflect contamination over different periods of the year. The highest concentrations in chicks were found in Djerba (Tunisia) with blood Hg values of (mean ± SD) 1.69 ± 0.51 μg g^{-1} dry weight (dw). Adults were most contaminated in Djerba and Dragonera (Balearic Islands, Spain) with blood Hg concentrations of respectively 3.78 ± 2.54 and 5.25 ± 3.73 μg g^{-1} dw. Trophic ecology was investigated using stable isotope analyses ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ as proxies of feeding habitat and diet), and showed that spatial variation in Hg was mainly driven by foraging habitat in both chicks and adults. Low Hg concentrations were related to the use of anthropogenic food sources. An effect of colony location was also found, suggesting spatial differences in local environmental pollution transfer up to seabirds. Our results also supported the use of $\delta^{34}S$ to discriminate between marine and continental foraging habitats in generalist seabirds. This study provides new insights onto the spatial distribution of Hg contamination in a widespread seabird, reporting some of the highest Hg values recorded for this species. Populations with highest concentrations are of potential concern regarding toxicological risks.

1. Introduction

Mercury (Hg) is a non-essential trace metal element naturally found in the environment, emitted in particular by volcanic eruptions and hydrothermal vents (Pirrone et al., 2010). It is also released in large amounts by anthropogenic activities, mainly during the combustion of

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fossil fuels and by artisanal and small-scale gold mining (Esdaile and Chalker, 2018; UN Environment Programme, 2019). Once in the atmosphere, Hg can be transported up to a year by atmospheric currents before being deposited onto land or water bodies (Streets et al., 2019) and therefore can be deposited in ecosystems a long way from the initial source. In aquatic environments, inorganic mercury (iHg) is transformed into methyl-mercury (MeHg) through biotic methylation reactions performed by microorganisms such as iron- and sulfate-reducing bacteria (Compeau and Bartha, 1985; Heimbürger et al., 2010). This organic form of Hg is highly toxic, highly bioavailable and strongly retained in biotic tissues (Rice et al., 2014). Consequently, MeHg accumulates in organisms over time and is biomagnified along food webs (Atwell et al., 1998; Harding et al., 2018; Ourgaud et al., 2018). Marine top predators, such as seabirds, thus have some of the highest levels of Hg concentrations among living organisms (Muirhead and Furness, 1988). Seabirds are long-lived species which tend to be philopatric to their natal and breeding colony, but with broad geographic distributions. They can therefore be contaminated differently depending on their location. These characteristics make it possible to study contamination over long temporal and spatial scales, such as that of an ocean basin (Carravieri et al., 2014a; Fort et al., 2014; Brown and Takada, 2017).

The Mediterranean Sea is a semi-enclosed, highly anthropized sea, making it prone to significant contamination from human populations along its coasts and from river discharges and run-offs. Indeed, the limited exchanges with the Atlantic Ocean increase the retention of persistent pollutants in its waters (Millot and Taupier-Letage, 2005). Furthermore, the Mediterranean Sea is described as an 'anomaly' due to unusually high Hg concentrations in organisms compared to other systems where seawater concentrations are similar; this has been attributed to the influence of temperature and oligotrophy (Cossa and Coquery, 2005; Chouvelon et al., 2018). However, to the best of our knowledge, a large-scale study of Hg contamination in seabirds has not yet been carried out in the Western Mediterranean basin. The few existing studies have focused on localized sites or small spatial scales, and on different avian tissues and species (e.g. Arcos et al., 2002; Sanpera et al., 2007a; Abdennadher et al., 2010; Ramos et al., 2013; Albertos et al., 2020; Costantini et al., 2020; Sánchez-Fortún et al., 2024).

Among seabirds, the yellow-legged gull (*Larus michahellis*) is the most common, wide-spread and abundant seabird species around the western Mediterranean (Vidal et al., 1998). It is therefore a valuable species for studying the spatial variation of Hg contamination along the coasts of the Mediterranean basin. Over the last hundred years, gulls have gradually shifted their diet towards easily accessible food sources, often of anthropogenic origin (Pedro et al., 2013; Oro et al., 2013). Their diet may consist of remains from fishing vessel discharges, marine invertebrates, terrestrial invertebrates or human waste from open dumps (Arizaga et al., 2013; Matos et al., 2018; Ramos et al., 2009). Access to fishing discharges gives them a source of demersal and mesopelagic fishes, that are prone to show high levels of Hg contamination (Arcos et al., 2002; Choy et al., 2009; Chouvelon et al. 2012). Previous studies have shown that Hg contamination of yellow-legged gulls can vary spatially (Ramos et al., 2013) and that pollution from industrial activities could be one of the causes (e.g., industrial zone, Tunisia: Abdennadher et al., 2010; chlor-alkali plant, Ebro Delta: Sánchez-Fortún et al., 2024).

Trophic ecology of birds is generally decisive in the observed spatial, temporal and inter-individual variations in Hg contamination, as this pollutant is mainly derived from food (Carravieri et al., 2014a; Albertos et al., 2020). To investigate the role of trophic ecology, the use of stable isotope analyses (SIA) has often been combined with ecotoxicological studies. Nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) stable isotopes values provide information on diet and foraging habitat, respectively (Hobson, 1987; Ourgaud et al., 2018). Sulfur ($\delta^{34}S$) stable isotopes values have been used recently as a complement to $\delta^{13}C$ to discriminate between terrestrial and marine habitats (Góngora et al., 2018; Binkowski et al., 2021).

In this context, the aim of the present study was to investigate spatial variation in Hg contamination in yellow-legged gulls around the Western Mediterranean basin. We evaluated Hg concentrations in both blood and feathers as these tissues can be sampled non-lethally and provide information on contamination at different temporal scales (Albert et al., 2019). More specifically, we aimed to: i) assess local contamination through the use of chick blood samples, chicks of yellow-legged gulls being fed only with prey from the vicinity of the breeding site (maximum 40 km; Oro et al., 1995); ii) investigate the local variation of toxicity risks in populations of adult gulls using blood and feather samples, as blood reflects the last few weeks of Hg contamination and feathers reflect longer term contamination (several months between molting sequences) (Albert et al., 2019). Both of these tissues can also be used to access information regarding their trophic ecology (diet and feeding habitat) with SIA to understand whether spatial variation in Hg is linked to differences in individual trophic ecology or to differences in environmental contamination (Forero and Hobson, 2003).

We predicted that individual Hg contamination would depend on the location of the colony, partly reflecting the heterogeneous distribution of MeHg in Mediterranean waters (Cossa et al., 2022) and the variable industrialization of coastal zones (Escoruela et al., 2018). We predicted that birds' feeding habitat and trophic position would explain inter-individual variability in Hg concentrations, with birds feeding at higher trophic levels and in more marine habitats being more heavily contaminated.

2. Material and methods

2.1. Sample collection and preparation

Fieldwork was carried out during the breeding seasons (March to June) of 2021 and 2022, in 17 yellow-legged gulls colonies in France, Spain and Tunisia (Fig. 1). Adult birds were caught on the nest during egg incubation, using a clapnet placed over the eggs. Chicks were captured by hand just prior to fledging (>1 month of age). Feathers ($n = 85$) and blood ($n = 134$) samples were collected from adults. Both tissues were also collected for chicks, but only blood ($n = 271$) was analyzed because of the strong correlation of Hg levels between the tissues (Binkowski et al., 2021). Body feathers were collected from the back and belly (approx. 3–5 feathers) and blood from the brachial vein under the wing (1–3 mL). In most cases, blood was centrifuged on site to separate red blood cells (RBC) from plasma. Some samples were preserved in 70% ethanol until analysis, while others were frozen directly. It should be noted that this difference in preservation does not affect subsequent chemical measurements (Hobson et al., 1997).

Blood samples preserved in ethanol had to be evaporated for about 48 h before being frozen. All samples were then freeze-dried for 24–48h, and homogenized. Whole blood (RBC + plasma) and RBC samples were processed identically. Hg in plasma is generally less than 10% of Hg in whole blood (Bond and Robertson, 2015). However, to ensure there was no bias due to the different sampling methods, we corrected the whole blood values to remove Hg possibly contained in plasma, using the higher percentage outlined in Bond and Robertson (2015), i.e., subtracting 8.8% of Hg in the whole blood samples (Table S1). These corrected values were used in the statistical analyses.

Body feathers were prepared by removing the calamus and the down, to have homogeneous samples. Prepared feathers were then cleaned in an ultrasonic bath in a 2:1 mixture of chloroform:methanol for 3 min, and rinsed in two successive methanol baths, to remove external contamination. They were then dried in an oven at 45 °C for 48 h, 3 feathers were pooled to reduce inter-feather variability and cut with stainless steel scissors to obtain a homogeneous fine powder. Mercury measured in feathers was assumed to reflect contamination over a longer time period (several months) than blood (several weeks), including the non-breeding season (Albert et al., 2019). Mercury is deposited in body feathers during the feather growth phase, which is believed to occur

Fig. 1. Map of the different colonies studied, with samples collected on chicks and/or adults. Projection ESPG:3035. Coordinates of the colonies can be found in Table 1.

once a year in gulls during the post-breeding period (Olsen and Larsson, 2003; Sanpera et al., 2007b) and is mainly due to the remobilization of stored Hg in internal tissues and organs (Goede and De Bruin, 1986; Braune and Gaskin, 1987). Body feathers, growing quickly over a short period of time, are small in size and reduce inter-feather variability in comparison to primary feathers (Braune and Gaskin, 1987).

2.2. Mercury analyses

Between 0.3 and 5 mg of dry material from each sample was analyzed in an Advanced Mercury Analyser spectrophotometer (Altec AMA 254). The detection limit of the AMA was 0.1 ng. The obtained concentrations are hereafter given in μg g^{-1} dry weight (μg g^{-1} dw). Two to three analyses were carried out per sample until the coefficient of variation between two measurements was <10%. The quality of analyses was checked using a certified reference material, TORT-3 (lobster hepatopancreas, National Research Council of Canada, which has a certified concentration of (mean ± SD) 0.292 ± 0.022 μg g^{-1} dw). Our TORT3 measurements averaged 0.290 ± 0.006 μg g^{-1} dw, with a recovery from the reference value of 99.2 ± 2.2% (n = 39). Mercury found in blood and feathers is predominantly MeHg (>90%, Bond and Diamond, 2009; Renedo et al., 2017). We therefore consider total Hg concentrations measured in birds as a proxy for MeHg, without differentiating between Hg species (organic or inorganic). Threshold values for Hg toxicity were extracted from Ackerman et al. (2016) (blood) and Chastel et al. (2022) (feathers). Risk classes were defined in adults from these values and converted from wet weight (ww) to dry weight (dw) considering 79.13% moisture content of blood (Eagles-Smith et al., 2008). Feather values were not converted to wet weight, as feather moisture was considered negligible (Stettenheim, 2000).

2.3. Stable isotope analyses

Stable isotope analyses of $\delta^{13}C$ and $\delta^{15}N$ in feathers and $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ in blood involved encapsulating an aliquot (0.2–0.8 mg) of samples in tin capsules. Isotopic measurements were carried out using an elemental analyser (Flash, 2000; Thermo Scientific, Milan, Italy) coupled to an isotope ratio mass spectrometer (Delta V Plus with a Conflo IV interface, Thermo Scientific, Bremen, Germany) to determine the relative abundance of isotopes in each sample. The instrument was calibrated using certified reference materials for isotopic values (caffeine USGS-61 and USGS-63 for C and N, silver sulfide IAEA-S2 for S). Results are expressed in δ, the deviation of the element's isotopic ratio from the isotopic ratio of an international standard (Vienna Pee Dee Belemnite for $\delta^{13}C$, atmospheric nitrogen (N_2) for $\delta^{15}N$ and Vienna Canyon Diablo Troilite for $\delta^{34}S$) according to the following equation:

$$\delta X = [(Rsample / Rstandard) - 1] \times 1000$$

where X (in ‰) is ^{13}C, ^{15}N or ^{34}S, and R is the ratio ($^{13}C/^{12}C$, $^{15}N/^{14}N$ or $^{34}S/^{32}S$), relative to standard values. The analytical precision was <0.10 ‰ for $\delta^{13}C$ and $\delta^{15}N$, and <0.25 ‰ for $\delta^{34}S$.

$\delta^{15}N$ values are indicative of the diet and the relative trophic status, with higher $\delta^{15}N$ values indicative of a higher trophic position due to ^{15}N enrichment along the food chain (Hobson et al., 1994). The $\delta^{13}C$ and $\delta^{34}S$ discriminate between marine and terrestrial origin of the diet, with higher values corresponding to a more marine diet (DeNiro and Epstein, 1978; Arizaga et al., 2013). Nevertheless, baseline stable isotopes of marine food webs are known to vary spatially (McMahon et al., 2013; Graham et al., 2010). Due to these baseline differences between Mediterranean regions (David Wells et al., 2021), $\delta^{15}N$ and $\delta^{13}C$ values were corrected using previously published $\delta^{13}C$ and $\delta^{15}N$ values in European pilchard (sardines) *Sardina pilchardus* from several Mediterranean locations. Sardines, despite not being one of the lowest trophic levels, remain at the same trophic level across different regions of the Mediterranean (Albo-Puigserver et al., 2016; Giménez et al., 2023). This allowed us to compare distant colonies showing extreme $\delta^{15}N$ and $\delta^{13}C$ values. Stable isotope values from sardines (Table S2) were subtracted from yellow-legged gull blood isotope values following Smith et al. (2021). As $\delta^{34}S$ values vary mostly between freshwater and seawater (Fry, 2002; St. John Glew et al., 2019), they were not corrected.

2.4. Statistical analyses

All statistical analyses were performed with R 4.2.2 (R Core Team, 2022). Data normality was checked using quantile-quantile plots (Q-Q plots). To examine inter-colony differences in Hg concentrations, we carried out descriptive mean comparisons. As Hg data tends to be skewed, log-transformed values improved the normality for parametric statistical tests. When data were normally distributed and sample sizes were similar, analyses of variance (ANOVAs) were performed, followed by post-hoc Tukey tests. Otherwise, non-parametric tests (Kruskal Wallis test and Bonferroni post-hoc test) were performed. A cut-off value of $\alpha <$ 0.05 was used for test significance. Some nests were sampled several times (different birds of the same family or over years), leading to

pseudo-replicates that could bias the analyses. To avoid this problem, we randomly selected only one chick per nest for the analyses. To compare life stages and sampling years, only colonies where both life stages and years had been sampled were considered. As the adults showed significantly higher Hg concentrations than the chicks (ANOVA: $F_{1,\ 258} = 60.03$, $p < 0.001$), the two life stages were separated in the analyses. As no significant difference was found between years (chicks, ANOVA: $F_{1,\ 36} = 0.05$; $p = 0.83$; adults, ANOVA: $F_{1,\ 23} = 2.42$; $p = 0.14$), data from the two years were grouped.

In order to investigate the drivers of Hg concentrations, generalized linear models (GLM) with a gamma distribution were built by discarding significantly correlated isotope values. Corrected Hg concentrations were defined as the dependent variable and corrected isotope values and mass (only available for colonies where feathers were sampled) as continuous explanatory variables. The best models were selected using the Akaike Information Criterion corrected for small samples sizes (AIC_c) (Burnham and Anderson, 2002), keeping the model with the lowest AIC (package AICcmodavg).

To compare chicks Hg concentrations with previously published data, we averaged several years of sampling from the literature, and converted feather Hg values to blood equivalent values using the regression between blood and feather Hg values in chicks of three gull species (*Larus argentatus*, *L. fuscus* and *L. marinus*): $\log Hg(blood) = 1.087 * \log Hg(feathers) - 1.478\ (Adjusted\ R^2 = 0.89)$, from Binkowski et al. (2021). The values reported from the literature can be found in the Supplementary materials (Table S3).

3. Results

3.1. Spatial variations of Hg in chick blood

Mercury concentrations in yellow-legged gull chick blood varied significantly between colonies (Kruskal-Wallis: $H_{13} = 93.2$; $p < 0.001$), with mean values ranging from 0.22 ± 0.16 μg g^{-1} dw at Urbino (Corsica) to 1.69 ± 0.51 μg g^{-1} dw at Djerba (Tunisia) (Table 1). Mercury concentrations at Djerba were significantly higher than any other colony (Bonferroni post-hoc test: $p < 0.05$). Some colonies exhibited moderate concentrations, some exhibited low concentrations and there were colonies with overlapping variability with the other groups (respectively group b, c and bc, Table 1). Two models had a $\Delta AIC_c < 2$ (Table 2). The first best model ($\Delta AIC_c = 0$) showed that $\delta^{34}S$ and colony were the most relevant explanatory variables for blood Hg concentrations, with Hg concentrations increasing with $\delta^{34}S$ (β±SE: 0.23 ± 0.03; CI: 0.18–0.29). The second best model included $\delta^{15}N$ (corrected) and colony as the most relevant explanatory variables, with Hg concentrations increasing with $\delta^{15}N$ (β±SE: 0.79 ± 0.09; CI: 0.61–0.97). Models were not averaged as $\delta^{34}S$ and $\delta^{15}N$ were correlated, and were both considered as plausible models. Models accounted respectively for 59% and 58% of the variance. To compare our data with those from previous studies, colonies and Hg concentrations in yellow-legged gull chicks were synthesized on a map (Fig. 2).

3.2. Spatial variations of Hg in adult blood and toxicity risks

Mercury concentrations in adult blood varied significantly among colonies (Kruskal-Wallis: $H_8 = 75.9$; $p < 0.001$), with mean values ranging from 0.46 ± 0.24 μg g^{-1} dw in Piana (Corsica) to 5.25 ± 3.73 μg g^{-1} dw in Dragonera (Balearic Islands) (Table 1). Model selection showed that $\delta^{34}S$ and colony were the most relevant explanatory variables for Hg concentrations in adult blood, explaining 79% of the variance (Table II). Blood Hg concentrations increased with $\delta^{34}S$ (β±SE: 0.26 ± 0.02; CI: 0.21–0.31). As such, we would expect an increase of approximately 0.2 μg g^{-1} dw of Hg concentrations for every 1 ‰ increase in $\delta^{34}S$ values. Among adults, 50.0% were in the "no risk" category (n = 67), 41.8% in the "low risk" category (n = 56) and 8.2% (n = 11) were in the "moderate risk" category (Djerba, n = 6 (4.5%); Dragonera, n = 5 (3.7%); Fig. 3a).

3.3. Spatial variations of Hg in adult feathers and toxicity risks

Feather samples from adults were only available for five colonies: Carteau, Frioul, Planasse, Piana (France) and Medes (Spain). Mercury concentrations in feathers did not vary significantly among colonies (ANOVA: $F_{4,80} = 2.125$; $p = 0.085$), with mean values ranging from 1.30 ± 0.61 at Carteau to 2.47 ± 1.77 μg·g^{-1} dw at Medes. Mercury concentrations were higher in the feathers (1.81 ± 1.77 μg g^{-1} dw) than in blood (0.96 ± 0.90 μg g^{-1} dw), with the exception of the colony of Carteau (feathers: 1.30 ± 0.61 μg g^{-1} dw; blood: 1.86 ± 1.10 μg g^{-1} dw). Model selection showed three best models, and the most parsimonious one was chosen as the best model (Table 2). In this model, $\delta^{15}N$ and mass were the most relevant explanatory variables, with Hg concentrations increasing with $\delta^{15}N$ (β±SE: 0.75 ± 0.13; CI: 0.53–0.99) and mass (β±SE: 0.002 ± 0.0007; CI: 0.0003–0.003). This model accounted for 47% of the variance.

Concentrations in adult feathers showed that 60.0% (n = 51) of birds could be classified as being at "no risk", 34.1% (n = 29) at "low risk" and 4.7% (n = 4) at "moderate risk". One individual from Frioul was in the "high risk" category, with a Hg concentration exceeding 9.14 μg g^{-1} dw (Fig. 3b).

4. Discussion

This study is, to the best of our knowledge, the first to examine Hg contamination in a seabird species at the scale of the Western Mediterranean basin. We demonstrate strong spatial variations with higher Hg concentrations measured in Tunisia (Djerba) and in the Balearic Islands (Dragonera), mainly driven by trophic ecology in both chicks and adults, as well as by colony location. The consumption of prey of marine origin (inferred from $\delta^{34}S$) and of high trophic level (inferred from $\delta^{15}N$) is likely to be linked to higher Hg concentrations (Ramos et al., 2013; Peterson et al., 2017). As a consequence of the observed Hg concentrations, more than 40% of adults were potentially at some risk of toxicity (i.e, above the "no risk" toxicity benchmark), with several adults falling into the « moderate risk » category at Djerba and Dragonera during the breeding period, and at Medes and Planasse during the non-breeding period. We wish to stress that in the present study, Hg concentrations measured in feathers were assumed to reflect contamination during a longer period than blood, including the non-breeding period. The molting pattern of yellow-legged gulls is still not fully understood (Arizaga et al., 2019), which could impact the period of Hg accumulation reflected by feather measurements. Future studies determining the molting sequence of yellow-legged gulls along with Hg excretion in feathers are nonetheless needed to fully validate this assumption.

4.1. Spatial distribution of Hg in chicks and adults

Chick blood Hg concentrations in the northern part of the Western Mediterranean basin were overall low in the colonies spread along the French Mediterranean coast, including Corsica, and in Melilla. Mercury concentrations in chick blood were higher in Djerba than in the other colonies. At this location, Hg concentrations were above 1 μg g^{-1} dw (average of 1.69 ± 0.51 μg g^{-1} dw) while gull chicks, worldwide, generally show values lower than this benchmark (e.g. Binkowski et al., 2021; Jouanneau et al., 2022). To the best of our knowledge, this is the highest value reported in yellow-legged gull chicks in the published literature (Abdennadher et al., 2010; Ramos et al., 2013; Zorrozua et al., 2020; Jouanneau et al., 2022). In adults, higher Hg blood concentrations were found notably in the sites of Dragonera and Djerba. Previous studies revealed that Dragonera yellow-legged gulls shifted their diet towards more marine sources after the closure of the landfill nearby

Table 1
Mercury concentrations in blood of yellow-legged gull chicks and adults at all colonies. Blood samples are red blood cells except for colonies marked with a *, which were whole blood samples. Colonies are ordered by increasing longitude. Values are arithmetic mean ± SD with in μg g^{-1} dw. Number of samples (n) are indicated before each life stage or tissue, except when it varied between measurements. Letters indicate significant differences within an age class (Bonferroni post-hoc test, $p < 0.05$). BI: Balearic Islands. Lat: latitude. Long: longitude. Mean values ± SD of stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ in ‰), for each colony and tissue of yellow-legged gull chicks and adults.

			Chicks					Adults								
			Blood					Blood					Feathers			
Colony	Lat	Long	n	Hg	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$	n	Hg	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$	n	Hg	$\delta^{13}C$	$\delta^{15}N$
Melilla* (Spain)	35.29	−2.94	20	0.44 ± 0.17^{bc}	−19.4 ± 0.4	10.6 ± 0.5	17.8 ± 0.8	–	–	–	–	–	–	–	–	–
Bosc* (Balearic Islands)	38.97	1.22	–	–	–	–	–	6	2.60 ± 1.58^{ab}	−22.1 ± 1.1	8.4 ± 0.8	8.4 ± 2.1	–	–	–	–
Penjats* (Balearic Islands)	38.82	1.41	–	–	–	–	–	4	1.59 ± 1.73^{abc}	−20.7 ± 1.4	8.6 ± 1.0	10.0 ± 3.7	–	–	–	–
Dragonera* (Baleraric Islands)	39.58	2.32	–	–	–	–	–	14	5.25 ± 3.73^{a}	−21.0 ± 0.5	9.9 ± 0.6	14.1 ± 3.0	–	–	–	–
Planasse (France)	43.09	2.99	–	0.42 ± 0.18 (22)^{bc}	−22.9 ± 0.6 (21)	8.3 ± 0.5 (21)	8.6 ± 1.8 (21)	19	0.46 ± 0.19^{c}	−22.3 ± 0.3	8.4 ± 0.3	7.3 ± 0.3	19	1.59 ± 1.37	−20.6 ± 1.0	10.1 ± 1.1
Sidrières (France)	42.90	3.01	20	0.79 ± 0.72^{b}	−22.3 ± 0.5	7.9 ± 0.4	10.5 ± 1.5	–	–	–	–	–	–	–	–	–
Medes (Spain)	42.05	3.22	30	0.46 ± 0.30^{bc}	−21.5 ± 0.6	8.4 ± 0.7	11.6 ± 2.6	25	1.05 ± 0.85^{bc}	−20.9 ± 0.6	10.5 ± 1.4	8.4 ± 1.1	20	2.47 ± 1.77	−20.9 ± 1.3	10.2 ± 0.8
Palavas (France)	43.54	3.96	6	0.69 ± 0.92^{bc}	−22.6 ± 0.5	8.5 ± 0.3	10.6 ± 1.7	–	–	–	–	–	–	–	–	–
Carteau (France)	43.38	4.86	38	0.27 ± 0.25^{c}	−23.0 ± 0.6	7.4 ± 0.5	8.9 ± 1.2	19	1.86 ± 1.10^{ab}	−21.0 ± 0.9	10.5 ± 1.4	10.5 ± 1.7	19	1.30 ± 0.61	−21.4 ± 1.1	9.5 ± 1.2
Frioul (France)	43.26	5.29	31	0.35 ± 0.29^{bc}	−22.5 ± 0.5	7.6 ± 0.3	9.3 ± 1.6	16	0.59 ± 0.47^{c}	−22.2 ± 0.4	8.5 ± 0.5	8.1 ± 0.7	16	1.96 ± 2.25	−20.9 ± 1.3	10.1 ± 1.8
Riou (France)	43.18	5.38	19	0.33 ± 0.31^{bc}	−22.4 ± 0.3	7.4 ± 0.3	10.1 ± 1.2	–	–	–	–	–	–	–	–	–
Porquerolles (France)	43.02	6.24	24	0.25 ± 0.13^{c}	−22.7 ± 0.5	7.7 ± 0.4	9.4 ± 1.7	–	–	–	–	–	–	–	–	–
Port Cros (France)	43.01	6.38	–	0.46 ± 0.24 (12)^{bc}	−21.8 ± 0.4 (9)	7.5 ± 0.4 (9)	12.2 ± 1.3 (9)	–	–	–	–	–	–	–	–	–
Pietra (France)	42.64	8.93	–	0.36 ± 0.06 (3)^{bc}	−22.5 ± 0.2 (2)	8.2 ± 0.01 (2)	13.4 ± 0.9 (2)	–	–	–	–	–	–	–	–	–
Piana (France)	41.37	9.23	–	0.86 ± 1.63 (13)^{bc}	−22.1 ± 1.0 (10)	7.12 ± 0.7 (10)	9.9 ± 1.9 (10)	11	0.51 ± 0.32^{c}	−22.4 ± 0.5	8.23 ± 0.2	9.78 ± 1.7	11	1.61 ± 1.07	−21.7 ± 0.8	9.89 ± 1.0
Urbino (France)	42.05	9.47	21	0.22 ± 0.16^{c}	−22.2 ± 0.4	8.21 ± 0.3	5.0 ± 0.8	–	–	–	–	–	–	–	–	–
Djerba (Tunisia)	33.81	10.85	20	1.69 ± 0.51^{a}	−18.3 ± 0.9	8.3 ± 0.4	14.1 ± 1.3	20	3.78 ± 2.54^{a}	−18.8 ± 1.6	9.03 ± 0.9	12.3 ± 2.7	–	–	–	–
Mean/Total			**271**	**0.50 ± 0.58**				**134**	**1.91 ± 2.30**				**85**	**1.81 ± 1.55**		

Table 2
Models explaining variations in Hg concentrations in the blood of chicks and adults and feathers of adults of yellow-legged gulls in the Western Mediterranean. K: number of parameters. AICc: Akaike Information Criterion corrected for small sample size. ΔAICc: difference in AICc of the model compared with the model with the lowest AICc. w_i: Akaike weight. McFadden's R^2: pseudo R^2 calculated from the null deviance and the residual deviance. $\delta^{15}N_c$: corrected $\delta^{15}N$. $\delta^{13}C_c$: corrected $\delta^{13}C$. Hg whole blood values were corrected (see Methods).

	Models	K	AIC_c	ΔAIC_c	w_i	McFadden's R^2
Chicks (blood) – GLM, gamma distribution, log link function	**Hg ~ $\delta^{34}S$ + colony**	**16**	**−71.53**	**0.00**	**0.68**	**0.59**
	Hg ~ $\delta^{15}N_c$ + colony	**16**	**−70.03**	**1.50**	**0.32**	**0.58**
	Hg ~ $\delta^{13}C_c$ + colony	16	−40.41	31.12	0.00	0.54
	Hg ~ colony	15	16.30	87.83	0.00	0.43
Adults (blood) – GLM, gamma distribution, log link function	**Hg ~ $\delta^{34}S$ + colony**	**11**	**227.19**	**0.00**	**1**	**0.79**
	Hg ~ $\delta^{13}C_c$ + colony	11	274.62	47.43	0	0.71
	Hg ~ $\delta^{34}S$	3	289.15	61.96	0	0.64
	Hg ~ $\delta^{15}N_c$ + colony	11	297.38	70.19	0	0.66
Adults (feathers) – GLM, gamma distribution, identity function	**Hg ~ $\delta^{15}N$ + colony + mass**	**8**	**199.06**	**0.00**	**0.53**	**0.53**
	Hg ~ $\delta^{15}N$ + colony	**7**	**200.50**	**1.44**	**0.26**	**0.51**
	Hg ~ $\delta^{15}N$ + mass	**4**	**200.99**	**1.93**	**0.20**	**0.47**
	Hg ~ $\delta^{15}N$	3	205.94	6.88	0.02	0.42

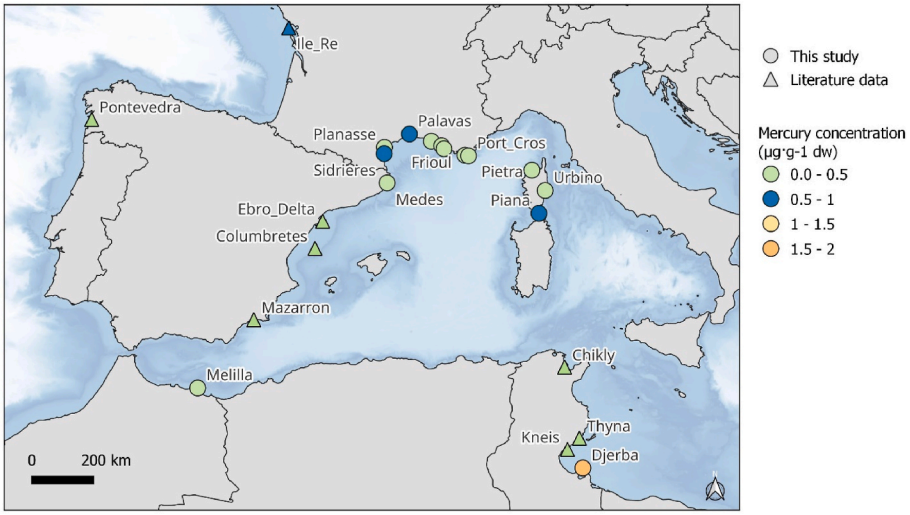

Fig. 2. An overall synthesis of Hg concentrations in blood equivalent of yellow-legged gull chicks in the Western Mediterranean and in two sites of the Atlantic Ocean for comparison. Circles indicate values from this study and triangles indicate values from previous studies. Projection ESPG:3035. Data from Otero and Fernández-Sanjurjo (2000), Abdennadher et al. (2010), Ramos et al. (2013) and Jouanneau et al. (2022). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

their breeding site (Payo-Payo et al., 2015). Higher $\delta^{34}S$ values in adults from Dragonera than at the other colonies located in Ibiza, in the Balearic Islands, where refuse in an open landfill was still available (Sanz-Aguilar, unpublished data), support the idea that a more marine-based diet at this colony makes birds prone to higher Hg contamination. Indeed, changes in avian Hg concentrations often result

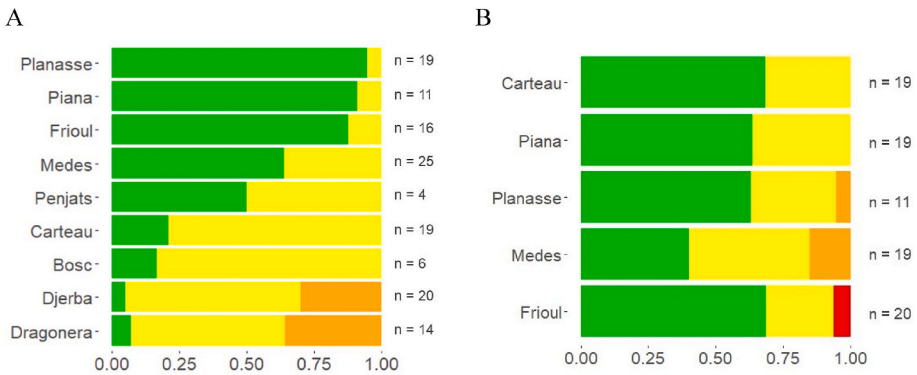

Fig. 3. Ranked overview (from lowest to highest risk) of the proportion of yellow-legged gull adults at risk for Hg negative effects by colony, categorized into four categories of risk based on blood (A) and feather (B) Hg effects from Ackerman et al. (2016); Chastel et al. (2022). Mercury concentrations in the following categories are expressed in µg g^{-1} dw. Blood: no risk <0.96 (green); low risk 0.96–4.79 (yellow); moderate risk 4.79–14.37 (orange). Feathers: no risk <1.62 (green); low risk 1.62–4.53 (yellow); moderate risk 4.53–9.14 (orange); high risk 9.14–10.99 (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

from dietary differences among individuals and populations (Carravieri et al., 2014a; Binkowski et al., 2021), with high trophic level prey showing higher Hg concentrations (Chouvelon et al., 2018; Bustamante et al., 2006). Foraging habitat can also be an important predictor of Hg contamination in seabirds (Ramos et al., 2013; Carravieri et al., 2017). However, in the present study, $\delta^{13}C$ did not explain spatial Hg patterns and measured concentrations in chicks. We hypothesize that in yellow legged-gulls, which can feed both on terrestrial and marine habitats, carbon isotopes are not sensitive enough to discriminate between prey of continental and marine sources, as showed in Ramos et al. (2013). On the contrary, $\delta^{34}S$ appeared to significantly and positively explain changes in Hg concentrations. This supports previous studies (Ramos et al., 2013; Góngora et al., 2018) emphasizing the importance of using $\delta^{34}S$ when studying the role of habitat, especially in coastal generalist seabirds such as gulls. The overall isotopic values of nitrogen, carbon and sulfur were low in both chicks and adults (except at Dragonera), suggesting a diet with a significant proportion of food of terrestrial origin at most sites (Ramos et al., 2013). The proximity of landfills around colonies along the French Mediterranean coast could facilitate gulls' access to terrestrial food sources during the breeding season that are less contaminated with Hg (Duhem et al., 2003). This has been previously studied in a few colonies such as Medes, where gulls use a high proportion of refuse from landfill sites for feeding, including pork, chicken and beef, as these easily accessible food resources have a high energy value (Ramos et al., 2009). Our study therefore extends the spatial scope of these findings to the scale of the Western Mediterranean basin. Although the use of anthropogenic food sources may reduce the exposure of gulls to Hg (Leonzio et al., 1986; Ramos et al., 2013), it may increase exposure to other toxic pollutants such as lead (Leonzio et al., 1986; Ramos et al., 2013; Vizuete et al., 2022) or plastics (Nono Almeida et al., 2023). Generalist seabirds with similar trophic behaviors might follow similar Hg contamination patterns.

Feather Hg concentrations did not statistically vary among colonies. Similarly, there were no major differences among colonies in either $\delta^{13}C$ or $\delta^{15}N$ values in feathers, whereas colonies differed in isotopic blood values. The isotopic values of feathers reflect the diet during its synthesis (Bond, 2010), i.e. after the breeding period. On the other hand, Hg in feathers represents a longer period, with the remobilization of Hg in internal tissues during feather synthesis (Braune and Gaskin, 1987). We lack information on Hg remobilization dynamics and molting pattern of seagulls to accurately determine the period represented by Hg measurements in feathers. The different incorporation periods of isotopes and Hg mean that it is impossible to conclude on the relationship between these two compartments (Bond, 2010). Nevertheless, the isotopic results here strongly suggest an overlap of wintering areas, which now needs to be confirmed by telemetry data. At the individual level, $\delta^{15}N$ was a strong driver of Hg concentrations in adult feathers, as explained previously for blood Hg values. Sampling adult feathers in more colonies would confirm if there is less variation in feather Hg values than in blood Hg values in the Western Mediterranean.

Multiple processes are responsible for building up Hg concentrations in biota, such as bioconcentration, bioaccumulation and biomagnification (Cossa et al., 2022); therefore, both diet and environmental contamination must be considered when explaining Hg levels found in wildlife. While gulls use a large proportion of refuse tips, they also rely heavily on marine resources (e.g. 50% in Medes, Ramos et al., 2009). On average, Western Mediterranean Hg values measured in gulls were higher than those previously found in Atlantic colonies (Otero and Fernández-Sanjurjo, 2000; Jouanneau et al., 2022). This confirms high values previously reported both in other seabird species such as Cory's and Scopoli's shearwaters (*Calonectris borealis* and *C. diomedea*, respectively; Escoruela et al., 2018; Costantini et al., 2020; Renzoni et al., 1986) and in Mediterranean marine species in general (Chouvelon et al., 2018; Cossa et al., 2022). This spatial difference has been attributed to the high bioavailability of MeHg in the Mediterranean Sea (Tseng et al., 2021) and a higher methylation rate of inorganic Hg than in Atlantic waters, likely due to warmer temperatures and oligotrophy (Cossa and Coquery, 2015; Chouvelon et al., 2018). Marine top predators, as bioindicators of Hg contamination of their environment (Burger and Gochfeld, 2004) can reflect spatial variation in environmental Hg (Tseng et al., 2021). At the scale of the Mediterranean Sea, MeHg dissolved in water is also known to be heterogeneously distributed, with higher surface water concentrations in the western Mediterranean basin compared to the Eastern basin; and within the latter, the southern part near the African coast showing higher dissolved MeHg concentrations than in the north (Cossa et al., 2022). Hence, the pattern of Hg contamination observed in gulls largely mirrors these environmental studies. Industralized zones with large inputs of contaminants into the waters could lead to higher contamination of the environment and associated food webs (Sanpera et al., 2000). The Djerba colony, for example, is located near Sfax, a large industrial zone. As currents spread industrial waste towards the south of this location, we hypothesize it could explain the higher Hg concentrations found in Djerba chicks compared to the rest of the Western Mediterranean Sea (Abdennadher et al., 2010; Béjaoui et al., 2019). However, studies investigating Hg values in fish and cephalopods on the Tunisian coast (Joiris et al., 1999; Mezghani-Chaari et al., 2011; Rjeibi et al., 2014) did not show consistently higher or lower values than those found in other Mediterranean sites (Llull et al., 2017; Capodiferro et al., 2022), but some mollusc species presented heavy metal concentrations above standards established by the World Health Organization and the Food and Agriculture Organization (Rabaoui et al., 2013). Additional sampling in areas where we found the highest Hg concentrations would therefore allow one to investigate this hypothesis in more detail, as many marine species might be impacted by toxic levels of Hg. Generalist seabirds such as gulls present strong inter-individual variability in their diet (Ceia et al., 2014; Mendes et al., 2018), with some studies showing sex-related differences in the diet of yellow-legged gulls (Calado et al., 2020). Sex was not analyzed in this study, but previous studies showed females of this species tend to have lower Hg concentrations than males (Albertos et al., 2020). This was also noted in other seabird species (Becker et al., 2002; Ramos et al., 2009 but see Carravieri et al. 2014b). Sex-specific differences have been attributed to either Hg excretion through egg-laying by females (Ishii et al., 2017) or sex-specific differences in foraging habitats (Carravieri et al., 2014b). Studies of sex-specific foraging habits are now required to determine the degree to which sex is an additional driver of Hg contamination.

4.2. Spatial distribution of risk in adult yellow-legged gulls

Mercury presents toxicological risks for birds, and these risks can be inferred from blood and feather Hg concentrations based on previous syntheses documenting Hg effects on various bird species (Ackerman et al., 2016; Chastel et al. 2022, and references therein). We found that about 50% of adult gulls were above the no risk level for adverse health effects in blood (0.96 μg g^{-1} dw) and 40% were above the no risk level in feathers (1.62 μg g^{-1} dw). The "low risk" category represents Hg concentrations where effects start to appear, whereas more substantial effects with negative consequences for health and reproduction occur in the "moderate risk" category (Ackerman et al., 2016). In Dragonera and Djerba, about a third of adults sampled were above the « moderate risk » benchmark in blood (4.79 μg g^{-1} dw). Moderate adverse health effects on seabirds encapsulate a variety of consequences such as a decrease in egg hatchability (Heinz et al., 2009; Braune et al., 2012), an increased probability of skipping breeding (Tartu et al., 2013) or a decreased probability of breeding successfully the following year (Goutte et al., 2014). Other possible effects include behavioral and physiological alterations such as decreased parental commitment to chicks and increased oxidative stress (Chastel et al., 2022 and references therein). However, all adults sampled were breeding birds, and this could constitute a bias in our study as non-breeding adults could show higher Hg concentrations preventing them from breeding (e.g., Tartu et al.

2013) or accessing the breeding grounds.

The toxicity thresholds used to assess the deleterious effects of Hg were calculated from adult data from numerous avian species (Ackerman et al., 2016) and are therefore only indicative of potential risks. Consequently, toxicity thresholds were not used for chicks in this study, but it is likely that Hg has greater effects on juvenile stages, particularly at the developmental level, given that Hg is an endocrine disruptor and strongly neurotoxic (e.g., Heinz, 1979; Wiener et al., 2002; Ackerman et al., 2024). Moreover, Hg sensitivity may also depend on the species (Heinz et al., 2009) and the effects have not been studied yet in yellow-legged gulls. Additionally, selenium (Se), a Hg antagonist with protective effects (Khan and Wang, 2009; Manceau et al., 2021) should be considered when assessing Hg toxicity risks in bird populations (Cruz-Flores et al., 2024), as high Hg concentrations in a bird could prove less toxic if Se concentrations are also high (Carravieri et al., 2017, 2020).

Previous studies also showed that Hg exposure during the non-breeding period in adult birds influenced breeding Hg concentrations in blood (Lavoie et al., 2014; Carravieri et al., 2023; Quillfeldt et al., 2022). This could potentially cause carryover effects on reproduction and fitness (Fort et al., 2014; Carravieri et al., 2023). As some populations could be more at risk during the non-breeding period, this could translate into negative effects during the next breeding season that would not be explained by Hg exposure during breeding only. There is still a lack of data to understand where gulls are exposed to Hg during the non-breeding period, as gull movements depend on their age and colony of origin (Souc et al., 2023), and also varies among individuals. The colony of Carteau surprisingly exhibited lower Hg concentrations in feathers than in blood, which could be due to different foraging behaviors or locations during the non-breeding period. This potential seasonal variation in contamination demonstrates the importance of evaluating risks for seabirds at different temporal scales. However, our study is limited by the small number of colonies where feathers were sampled. To extend these findings to the whole Mediterranean Sea, sampling and analysis of body feathers at other colonies is needed.

5. Conclusions and perspectives

This study gives us new insights on the spatial distribution of Hg in a widespread and abundant seabird species, the yellow-legged gull. We found significant variation in Hg concentrations across the western Mediterranean basin, with low Hg concentrations in the northern part, and higher concentrations in southern Tunisia and the Balearic Islands. Mercury contamination was driven by trophic ecology, i.e. the use of marine resources by birds, and likely by local contamination. The use of $\delta^{34}S$ in this study showed a better discriminating power for the feeding habitat of gulls than traditional carbon stable isotopes. Our results raise concern for risks faced by both chicks and adults in these colonies, but also for the potential impacts of Hg contamination in other species sharing these environments. We used yellow-legged gulls as a bio-indicator species, but marine species using similar food sources could also be impacted such as other seabirds and predatory fish. This work therefore highlights geographic areas needing further investigation in the Western Mediterranean. Other factors should also be considered to assess pollutant concentrations in gulls, such as the influence of sex and year-round movements, especially during the non-breeding period. Future studies using tracking data to determine the feeding areas of gulls during this period should improve our knowledge on the sources of this pollutant.

CRediT authorship contribution statement

Laura Patier: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis. **Paco Bustamante:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Conceptualization. **Karen D. McCoy:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization. **Gaël Guillou:** Writing – review & editing, Methodology, Investigation. **Abdessalem Hammouda:** Writing - review & editing, Resources. **Carole Leray:** Writing – review & editing, Resources. **Gonzalo Fernando Martínez Salcedo:** Writing – review & editing, Resources. **Ana Payo-Payo:** Writing – review & editing, Resources. **Gauthier Poiriez:** Writing – review & editing, Investigation. **Raül Ramos:** Writing – review & editing, Resources. **Ana Sanz-Aguilar:** Writing – review & editing, Resources. **Slaheddine Selmi:** Writing – review & editing, Resources. **Giacomo Tavecchia:** Writing – review & editing, Resources. **Marion Vittecoq:** Writing – review & editing, Resources. **Jérôme Fort:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.124992.

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