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Relating trophic ecology and Hg species contamination in a resident opportunistic seabird of the Bay of Biscay

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Running head: Relating trophic ecology to Hg contamination

ABSTRACT

Methylmercury (MeHg) is the most bioavailable and toxic form of the globally distributed pollutant Hg. Organisms of higher trophic levels living in aquatic ecosystems have potentially higher concentrations of MeHg. In this work, we analysed both MeHg and inorganic Mercury (Hg(II)) concentrations from dorsal feathers of chicks from ten colonies of Yellow-legged Gull (*Larus michahellis*) in the south-eastern part of the Bay of Biscay. Overall, we detected a high mean MeHg concentration that, however, differed among colonies. Additionally, based on stable isotopes analysis ($\delta^{13}C$ and $\delta^{15}N$) and conducting General Linear Mixed Models, we found that chicks which were mostly/mainly fed with prey of marine origin had higher levels of MeHg. We propose Yellow-legged Gull as a reliable biomonitor for Hg species, as it is easy for sampling and in compliance with the Minamata convention on Mercury.

Keywords: biomonitor, diet, marine prey, mercury species, seabird, stable isotopes

1. INTRODUCTION

Mercury (Hg) is a globally distributed pollutant with severe impacts on ecosystems and human health (e.g. Eisler, 1987; Wolfe et al., 1998; Tan et al., 2009; Podar et al., 2015). It is a toxic metal with very adverse effects on wildlife, and on birds in particular, including physiological, neurological, behavioural and reproductive effects (Eisler, 1987; Scheuhammer, 1987; Ackerman, 2016a; Evers, 2018). It comes from both, anthropogenic and natural sources (Zhang et al., 2013) and its toxicity is related to its molecular speciation (Clarkson 1998; Renedo et al., 2017). Methylmercury (MeHg) constitutes the most bioavailable and toxic form of Hg to wildlife (Ullrich et al., 2001; Scheuhammer et al., 2007; Zhang et al., 2013) and, because it can be bioaccumulated and biomagnified through the food web, exposure to wildlife and humans occurs mainly via the consumption of organisms contaminated with this compound (Mason & Benoit 2003; Driscoll et al., 2007; Liu et al., 2008; Driscoll et al., 2013). The conversion of Hg to MeHg mostly occurs under anoxic conditions in aquatic systems (Driscoll et al., 2007; Cossa et al., 2009; Driscoll et al., 2013). Hence, species occupying higher trophic levels and either inhabiting freshwater or marine ecosystems and/or consuming prey from these habitats would be more exposed to this contaminant (Bargagli et al., 1998; Wiener et al., 2003; Scheuhammer et al., 2007; Goutte et al., 2014; Carravieri et al., 2014).

Seabirds have been shown to be appropriate organisms to evaluate Hg contamination in marine environments (Thomson et al., 1998; Burger & Gochfeld, 2004; Braune, 2007; Bond et al., 2015). Besides their position at the top of the trophic web, they are also long-lived, reinforcing the bioaccumulation and thus, mercury toxicity (Scheuhammer et al., 2007). Measuring the concentration of Hg species (MeHg and Hg(II)) in top predators not only provides insights into the degree of contamination in an ecosystem or through a trophic chain in given ecosystem, but also whether such concentrations are related to predators' trophic ecology. In other words, it is important to know not only whether Hg species concentrations are low or high, but also to identify the main Hg source(s).

The current socio-economic model provides a high amount of food subsidies (including fish discards, as well as organic garbage in landfills) that become available for those species with high trophic plasticity, such as the Yellow-legged gull. The occurrence of Hg species in such food subsidies is still a matter of concern, because bird populations that highly depend on these resources might be exposed to abnormally high concentrations of pollutants, thus falling into ecological trap with long-term negative demographic effects. Many gulls are able to feed on a broad range of prey, from marine to terrestrial food taken from landfills (Ramos et al., 2009; Washburn et al., 2013; Zorrozua et al., 2020a). In principle, it can be stated that those individual birds foraging on a higher proportion of marine prey would also show higher Hg concentrations (Wiener et al., 2003; Ramos et al., 2013; Peterson et al., 2017). As compared to other seabirds, many gulls also exploit other habitat types, including landfills, and they take benefit also from foraging on food subsidies, such as fish discards or organic garbage. Accordingly, they are good models to test for the presence of Hg species in food subsidies of a broad range of habitats, i.e. marine or landfills.

The Bay of Biscay is an important bird marine area in Europe, used by hundreds of thousands of seabirds as a corridor between breeding quarters in northern Europe and the tropical/southern Atlantic Ocean. Furthermore, local resident gulls depend on these waters where they spend the whole life. From an environmental point of view, Hg pollution in seabird populations through the south-eastern part of the Bay of Biscay remains largely unknown. The Yellow-legged Gull (*Larus michahellis*) is the most important seabird species breeding in the Bay of Biscay and it is fairly distributed along the Cantabrian coast. It is an opportunistic gull that exploits many foraging habitats, including anthropogenic origin landfills and fish discards (Arizaga et al., 2013, Arizaga et al., 2017; Zorrozua et al., 2020a).

Traditionally, Hg concentration in birds is assessed using blood, eggs or feather samples (Braune, 1987; Thomson et al., 1998; Bond & Diamond, 2009; Akearok et al., 2010; Hebert et al., 2011; Renedo et al., 2018). As compared to the blood and eggs, the use of feathers allows a less-invasive sampling and, moreover, feathers reflect Hg values accumulated in a longer period as compared to the other two tissues (Bearhop et al., 2000). Furthermore, feathers Hg content remain stable and hence the samples can be easily stored to be analysed even years later (Applequist et al., 1984; Thompson & Furness, 1989; Scheuhammer et al., 2007). Hg concentrations have been found to vary between age classes (adults vs. chicks), as well as among different feather types (Caldwell, 1999; Bearhop et al., 2000; Pedro et al., 2015; Peterson et al. 2019). Body feathers have been reported to show less variation in Hg than flight feathers (Furness et al., 1986), allowing more reliable comparisons. Moreover, the feathers allow inferring trophic ecology by analysing some chemical markers such as C and N stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) (Hobson et al., 1994). $\delta^{13}C$ is a reliable isotope to identify the foraging habitat, i.e. higher values of $\delta^{13}C$ have been related to more offshore marine foraging habits (Hobson et al., 1994), whereas $\delta^{15}N$ acquires higher values with increasing trophic levels, so this is a suitable isotope to assess consumer position within the trophic network (Schoeninger & DeNiro, 1984; Hobson et al., 1994; Forero & Hobson, 2003). Mixing models (SIAR; Parnell et al., 2008) allow inference about consumed prey categories by combining both $\delta^{15}N$ and $\delta^{13}C$.

In this work, we aimed to evaluate the relationship between the trophic ecology and the levels of Hg, as well as the suitability of the Yellow-legged Gull as biomonitor of Hg contamination. For that, Hg levels (inorganic and methylmercury) have been determined in 10 colonies of Yellow-legged Gull in the Bay of Biscay, given that these colonies show different trophic preferences.

2. MATERIALS AND METHODS

2.1.Samples and data collection

Sample collection was carried out in ten Yellow-legged Gull colonies situated along the coast of the south-eastern part of the Bay of Biscay (Fig. 1). All colonies are situated within an area of 135 km in straight line, holding a global population of ca. 1850 adult breeding pairs (census done in 2017; Zorrozua et al., 2020b). During the breeding period (June) of 2016 and 2017, ten chicks per colony and year were sampled and ringed at the age of ca. 20 days. A random sample of ten chicks per colony was reported to be enough to catch the inter-individual variability of Hg within a colony (Zabala et al., 2019). In these chicks their tarsus length was measured (as a surrogate of their body size; Jordi & Arizaga, 2016) and ca. 5-10 half- to fully-grown (but never pin or feathers just starting to emerge) dorsal feathers were taken for Hg and stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$).

2.2. Feathers preparation

The feathers were washed in a 1M NaOH solution and dried at 60ºC. Afterwards, they were homogenised into a fine powder using a cryogenic impactor mill (Freezer/mill 6750-Spex, Certiprep) that operates at liquid nitrogen temperature.

2.3. Stable isotopes analysis

Sub-samples of ca. 0.3 mg (for $\delta^{13}C$ and $\delta^{15}N$) were put in tin capsules for combustion to carry out the isotopic analysis by elemental analysis-isotope ratio mass spectrometry (EA-IRMS) with a ThermoFinnigan Flash 1112 coupled to a Delta isotope ratio mass spectrometer via ConFlo III interface. Stable isotope values were calculated as $\delta X = [(R_{sample} / R_{standard}) - 1] \times 1000$, where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. IAEA standards were applied every 12 samples to calibrate the system. Stable isotope ratios were expressed in the standard δ notation relative to Vienna Pee Dee Belemnite ($\delta^{13}C$) and atmospheric N_2 ($\delta^{15}N$). Standard replicates indicated analytical measurement errors of ≤ 0.1‰ and ≤ 0.3‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. Analyses were done at the Centres Científics i Tècnics (CCiT) at the University of Barcelona.

2.4. Hg speciation analyses

Extractions of Hg species (MeHg and Hg(II)) from feathers were carried out with an Explorer focused microwave system from CEM Corporation (Mathews, N.C., USA) with stirring. All the samples were extracted according to the same method. 200mg of feathers were extracted with 5 mL TMAH. 10µL to 200µL of extracts of each samples, were weighted in 4mL of buffer solution (HAc/NaAc, pH=4). After pH adjustment at 4 with HCl, 100µL of sodium tetraethylborate (NaBEt4) at 20% was added onto 2 to 6mL isooctane to derivatize Hg species. Organic phase was recovered after 5min of manual shaking and analyzed in triplicate by GC-ICP-MS. A commercial GC–ICP-MS interface (Silcosteel®, 0.5m length, inner I.D. 0.28mm and O.D. 0.53mm, outer i.d. 1.0mm and o.d. 1.6mm, Thermo Fisher Scientific, Franklin, MA, USA) was used to couple a Thermo Electron gas chromatograph (Trace) to a Thermo X2 series ICP–MS (Thermo Fisher Scientific, Waltham, MA, USA). Column is a MTX®-1 Silcosteel® (30m x 0.53mm x 1µm) which have a crossbond® 100% dimethylpolysiloxane stationary phase (Restek, Bellefonte, P.A., USA). A volume of 2µL of sample was introduced in splitless mode at 250°C. Temperature program used for the chromatographic separation was: 1 min at 60°C, temperature gradient from 60°C to 280°C at 60°C/min and 1 min at 250°C. Carrier gas was helium with a flow of 25mL/min and make-up gas was argon with a flow of 300mL/min. ICP-MS parameters used for analysis were: nebulizer, plasma and auxiliary flows 0.6, 1.5 and 0.9 L/min respectively, plasma power 1250W, Hg isotopes 198, 199, 200, 201 and 202 with dwell time of 25ms and Tl isotopes 203 and 205 with dwell time of 5ms. ICP-MS optimization was conducted with an Internal standard solution from Analytika (Prague, Czech Republic). Simultaneous introduction of Tl permitted to check mass bias during analysis. Accuracy was assessed by analysing the reference material RM IAEA-86 (Human hair): 0.258 ± 0.011 µg/g dw for MeHg and 0.315 ± 0.020 µg/g dw for Hg(II). Good agreement with certified values was obtained with recoveries of 104 ± 10 % and 90 ± 8 % for MeHg and Hg(II), respectively. Low detection limits were determined with 0.05 and 0.08 ng Hg/g dw for MeHg and Hg(II), respectively.

2.5. Statistical analyses

The variables used in this work were inorganic mercury (Hg(II)), methylmercury (MeHg) and total mercury concentrations (hereafter HgT) and, as well as the proportion of MeHg over the full amount of Hg in a sample (hereafter, Prop.MeHg). For the analysis mercury data were log-transformed to better adjust to a normal distribution.

First of all we explored to what extent Hg(II), MeHg and Prop.MeHg varied spatially and temporally; we used for that two-way ANOVAs on Hg(II), MeHg or Prop.MeHg as object variable, with colony and year as factors. Similar ANOVAs were done to test for the same effect on $\delta^{13}C$ and $\delta^{15}N$ signatures.

Second, to test for the effect of diet on HgT, MeHg or Prop.MeHg, we conducted General Linear Mixed Models (GLMM) with a linear link function with $\delta^{13}C$, $\delta^{15}N$ and tarsus length as covariates, and colony and year as random factor [R notation: HgT / MeHg / Prop.MeHg ~ $\delta^{13}C$ + $\delta^{15}N$ + *tarsus* + (1|*colo*) + (1|*year*)]. HgT and MeHg variables were log transformed to obtain a normal distribution, as some previous analysis suggested a better model fit. This first saturated model was run using the 'dredge' function provided by the packcage MuMIn (Barton, 2018) to obtain a best-parsimonious model which, with the lesser amount of possible parameters, may fit better to data. Model selection was conducted using the small-sample size corrected Akaike values (Akaike, 2011). Models differing in less than 2 AICc values were considered to fit to the data equally well. When we had two or more best-candidate models, these were averaged in order to obtain more representative parameter estimates.

All statistical analyses were done in R 3.5.1. (R Development Core Team 2011).

3. RESULTS

Overall, we detected Hg(II) and MeHg geometric mean values of 0.374 µg/g dw (95% CI: 0.306, 0.457) and 2.765 µg/g dw (95% CI: 2.554, 2.994), respectively. The Prop.MeHg over HgT reached 85 ± 9 % (arithmetic mean ± SD; Table 1) and ranged from 75 ± 9 % in Lekeitio (2016) to 96 ± 2 % in Getaria (2017). However, these values were very variable and were found to differ significantly among our studied colonies and years (MeHg: colony, $F_{9,123} = 3.23$, $P = 0.002$; year, $F_{1,123} = 13.97$, $P < 0.001$; Hg(II): colony, $F_{9,123} = 9.14$, $P < 0.001$; year, $F_{1,123} = 43.78$, $P < 0.001$; Prop.MeHg: colony, $F_{9,123} = 17.81$, $P < 0.001$; year, $F_{1,123} = 44.24$, $P < 0.001$; Fig. 2). Getaria was the colony with the highest MeHg (and HgT concentrarions) in 2016 with 4.816 µg/g dw geometric mean values (95% CI: 3.759, 6.169) and it was also the colony with the highest Prop.MeHg, 96 ± 2 % in 2017. The lowest values were estimated for Punta Lucero and Santa Clara colonies in 2017, with geometric mean MeHg values of 1.890 µg/g dw (95% CI: 1.388, 2.572) and 1.868 µg/g dw (95% CI: 1.355, 2.575), respectively. MeHg and HgT were highly correlated ($r = 0.97$, $P < 0.001$, 95% CI: 0.96, 0.98), whereas Prop.MeHg and HgT were not so correlated ($r = -0.37$, $P < 0.001$, 95% CI: -0.51, -0.21; Fig. 3).

With regard to stable isotopes, mean values of -19.37 ± 0.85 and 12.58 ± 1.08 were obtained for $\delta^{13}C$ and $\delta^{15}N$, respectively (Table 1, Fig. 4). As for Hg values, a high variation among colonies and years was detected ($\delta^{13}C$: colony, $F_{9,123} = 6.15$, $P < 0.001$; year, $F_{1,123} = 5.16$, $P = 0.025$; $\delta^{15}N$: colony, $F_{9,123} = 8.77$, $P < 0.001$; year, $F_{1,123} = 15.84$, $P < 0.001$).

Regarding the relationship of MeHg and HgT values to diet, we obtained a total of three (MeHg) or two (HgT) models that fitted to the data equally well (Table 2). The averaged model provided, anyway, a positive (significant) effect of $\delta^{15}N$ on either MeHg or HgT, as well as a significant negative effect of tarsus length on MeHg or HgT (Table 3). In contrast, neither $\delta^{15}N$ and $\delta^{13}C$ nor tarsus length had a significant effect on the Prop.MeHg (Table 3).

4. DISCUSSION

This is the first study where both, inorganic and methylmercury have been assessed for a number of colonies of the same species in the south-eastern part of the Bay of Biscay. Total mercury concentrations were, surprisingly, much higher (up to a mean of 6.124 µg/g dw in one colony) than what has been reported in other gulls (Szumilo-Pilarska et al., 2017) or seabird species (Carravieri et al., 2016) in some other areas. For several gull species up to 3.023 µg/g dw HgT mean values in adult body feathers were reported (Szumilo-Pilarska et al., 2017), up to 1.880 µg/g dw for penguin species (Carravieri et al., 2016) and up to 19.700 µg/g dw in petrels (Carravieri et al., 2014). However, these species have different foraging strategies and besides, variation in age and type of feathers sampled make sometimes difficult to compare values. Interestingly, quite similar concentrations to this works' results were found for Audouin's gull chicks in the western Mediterranean (Sanpera et al., 2007), with the highest values obtained for the colony in the Ebro Delta, 5.090 µg/g dw. In that case, the high concentration found in this colony was partly attributed to the anthropogenic mercury inputs in the area. In this study, Getaria (2016) was the colony with the highest HgT concentration with 5.861 µg/g dw, whereas Santa Clara (2017) was the one with the lowest values with 1.952 µg/g dw. Furthermore, we can think that, due to bioaccumulation and to feathers excreting Hg ingested between moults too (from the body pool; Furness et al., 1986; Thomson et al., 1998), adults still would have higher concentrations than chicks, so Hg(II) and MeHg values still would reach higher values. Overall, these values would indicate a high concentration of both Hg(II) and MeHg within the region. Interestingly, Prop.MeHg ranged from 75 % to 96 %, varying significantly among colonies situated close to each other. This variation has been reported before among species (Mallory et al., 2015), but it is unknown for us whether it has been found for colonies of the same species located so near. Still, for several seabird species in the Southern Ocean, MeHg proportion was never below 90 % (Renedo et al., 2017). Authors in this work also found that the higher the concentration of HgT, the lower the proportion of MeHg. Within our samples, however, MeHg and HgT concentrations were positively correlated.

Some of our individuals had Hg concentrations above 5,000 μg/g dw, a value found to reduce the reproductive output (e.g. reduced hatch of eggs and sterility; NAS, 1978). Overall, the Yellow-legged Gull population in the south-eastern part of the Bay of Biscay is stable, only decreasing in some colonies (Arizaga et al., 2009; Galarza et al., 2015; Juez et al., 2015), though this decline is majorly attributed to dramatic food shortage, e.g. due to landfill closures (Galarza et al., 2015). The productivity of all these colonies still remains to be studied in detail, and it should be investigated to what extent the very high concentrations of Hg species have a significant impact on any of the reproductive phases and, finally, on productivity, as well as on other physiological, neurological or behavioural aspects.

We found that birds with higher δ^{15}N values had higher HgT and MeHg concentrations. As δ^{15}N values increase in higher trophic levels, it can be concluded that chicks provided with prey situated at a higher trophic position are exposed to higher concentrations. Previous work in this area (Arizaga et al., 2013) indicates that higher δ^{15}N values point to more marine prey. Thus our results suggest that the relation found among MeHg and THg values and isotope signatures could indicate that feeding more on marine prey would be related with higher Hg concentrations, in accordance with other studies (Santos et al., 2017). Thanks to other studies (Arizaga et al., 2010) we know that this gull population feeds on both natural marine prey (captured near the coast) and fishing discards (which are not necessarily from the coast). Therefore, the Hg pollution source is still a bit diffuse and future studies where the origin of Hg could be better determined would be interesting. Overall, the Hg values found are high and according to the stable isotopes have a marine origin, thereby it can be concluded that the south-eastern Bay of Biscay has high Hg concentration. A possible explanation could be that several rivers from industrialised areas end in this bay and although their heavy metals concentration has decreased in the last years, mercury persists as a legacy pollutant. Moreover, it would be interesting to determine whether Hg has been accumulated in the deep sea. In this sense, demersal fish have been found to have higher levels of mercury than epipelagic fish (Arcos et al., 2002) and Chouvelon et al. (2012) reported particularly high Hg concentrations for deep-sea species in the Bay of Biscay. Thus, considering that gulls' potential deep-sea prey would probably come from fishing discards, European Policies aimed at eliminating fishing discards (European Union, 2013) might help to reduce Hg concentration values found in these gulls.

Our models related MeHg and THg values with tarsus length, which could be used as an age index (Jordi & Arizaga, 2016), hence older chicks would present lower Hg concentrations. This would fit with the fact that females allocate Hg in produced eggs (Becker & Sperveslage, 1989; Lewis et al., 1993; Ackerman et al., 2011; Ackerman et al., 2020), thus immediately after hatching chicks have high Hg concentrations. As the chick gains mass (the Hg dilutes in the body burden) and the newly growing feathers allocate Hg, Hg concentration in blood decreases (Hg ingestion is not sufficient to compensate Hg dilution; Ackerman et al., 2011). Indeed, the distal part of the feather has been found to contain higher Hg values compared to the proximal part (Burger & Gochfeld, 1992; Peterson et al., 2019), hence fully grown feathers present lower Hg concentration than the partially grown ones. Therefore, it is not until chicks are completely fledged that Hg concentration start to increase with age (Ackerman et al., 2011). In this work the sampling was carried out before chicks were able to fly, that is, before their feathers were fully developed, so our model results would be in accordance with previous knowledge.

The high number of Yellow-legged Gull sampling places (colonies), their broad distribution (even almost evenly spaced), the high number of individuals in each colony and their relatively easy accessibility are, overall, good a priori elements to consider the Yellow-legged Gull suitable as a biomonitor. Moreover, the species was observed to seemingly capture potential spatial variability of Hg(II)/MeHg concentrations, and their relationship with trophic ecology. Additionally, feathers can be collected non-invasively, they are more chemically and physically stable than blood, enabling to store them for longer. Lewis and Furness (1991) stated that the proportion of Hg excreted with feathers in relation to body burden was independent of the dose they administered in Black-headed

Gulls (*Chroicocephalus ridibundus*). Although this assessment should be confirmed for Yellow-legged Gull, it would allow us to make estimations of Hg exposure, or at least to evaluate potential changes. The studied population is resident and recent analysis based on GPS devices have shown that individuals do not travel high distances far into the sea, overall entering a maximum of 25 km into this habitat (Arizaga et al., 2017; Zorrozua et al., unpublished). However, the consumption of fish discards may entail consumption of prey captured further in the sea, being difficult to certainly attribute Hg exposure to the nearby area. An aspect to consider in Hg concentration assessment is the high variation found among colonies situated relatively near from one another, which suggests that the use of individuals of a single colony may give us biased information on Hg pollution. Trophic variation was also found in the colonies studied in this work, reflecting the trophic specialization existing among colonies (Zorrozua et al., 2020b). Hence, samples from different colonies are needed to obtain complementary and more reliable bioindicator values.

Some works, however, advise not to use chicks' fully grown feathers as a biomonitoring tool on Hg pollution, since Hg concentrations in chicks' internal tissues may rapidly change (Ackerman et al., 2016a). These authors, by contrast, recommend taking down feathers. However, analysing eggs is more aggressive for the population and need a high sample size (Ackerman et al. 2016b), a protocol ethically impossible to be implemented in a number of our too small colonies. Due to the high variability in Hg concentrations between different tissues, it would be interesting for the future to determine the magnitude of such variability within our Yellow-legged Gull population, since this would permit us to develop a better long-term Hg biomonitoring protocol within the region. Blood and feathers may provide complementary information at different temporal scales of Hg exposure in adult birds, as both blood and first synthesised feathers from chicks represent similar periods of Hg dietary intake (Renedo et al., 2018; Albert et al., 2019).

According to such results, we consider that the Yellow-legged Gull can be incorporated as a bioindicator in accordance to the Minamata convention on Mercury of the UN (UNEP, 2013). Specifically, Article 19 of this convention refers to the need to make an effort in assessing geographically representative Hg values, including the evaluation on bird populations, among other species. Additionally, the combined analyses of stable isotopes and Hg species and, probably, other complementary tools such as GPS-tracking of given individual birds (Carravieri et al., 2018) are called to contribute to identify Hg sources, hence polluted sites/habitats, thus meeting with Article 12 of this convention: "each party shall endeavor to develop appropriate strategies for identifying and assessing sites contaminated by mercury or mercury compounds".

In conclusion, high Hg exposure has been detected in the area by using Yellow-legged Gull population and it is one of the few studies that provide the relative proportion of both Hg species [MeHg, Hg(II)]. We propose this species' chick feathers as bioindicator to complement monitoring on Hg exposure in the Bay of Biscay, helping to detect Hg pollution beyond the static sampling sites that could have been established and in compliance with the Minamata convention on Mercury.

AUTHORS' CONTRIBUTIONS

NZ and JA conceived the ideas and designed the methodology; NZ, AA, IC, BD, AE, AG, JH and EM collected the data; NZ, MM, CS and JA analysed the data; NZ and JA led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

ACKNOWLEDGEMENTS

Thanks to the people who collaborated in the field work, specially the members of the Txingudi Ringing Station, forest rangers of Gipuzkoa, J. Menéndez and J. López. This work was financially supported by ERDF (European Regional Development Fund) and AEAG (Agence de l'Eau Adour-Garonne) grants in the framework of the MICROPOLIT project. The Basque Government, Gipuzkoa Administration and the Donostia City Hall partly funded this research. Partial funding was obtained from Project CGL2016-80963-R (AEI/FEDER, UE). The Administrations from Aquitaine, Gipuzkoa, Bizkaia and Cantabria gave permissions to collect feather samples in the colonies. N. Zorrozua benefited from a pre-doctoral fellowship from the Basque Government. Two anonymous referees provided valuable comments that helped us to improve an earlier version of this work.

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Table 1. Isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$ (mean ± SD), and the concentration of MeHg and Hg(II) (geometric mean, 95% confidence interval) obtained from dorsal feathers of Yellow-legged Gull chicks in 10 colonies situated across the south-eastern part of the Bay of Biscay. Colonies named as in Fig. 1.

Colony code	Year	Sample size	$\delta^{13}C$	$\delta^{15}N$	MeHg (µg/g dw)	Hg(II) (µg/g dw)
CAS	2017	10	-19.7 ± 0.4	12.1 ± 0.6	2.483 (2.024-3.047)	0.587 (0.487-0.708)
LUC	2017	10	-20.3 ± 1.3	11.3 ± 1.2	1.890 (1.388-2.572)	0.100 (0.075-0.132)
BIL	2016	8	-19.6 ± 0.3	11.9 ± 0.6	2.735 (2.137-3.501)	0.799 (0.630-1.012)
IZA	2016	10	-19.9 ± 0.5	11.5 ± 0.7	2.492 (1.918-3.239)	0.650 (0.471-0.898)
	2017	10	-19.4 ± 0.8	12.4 ± 0.9	2.523 (1.738-3.663)	0.583 (0.374-0.910)
LEK	2016	10	-19.0 ± 0.7	12.6 ± 1.1	3.405 (2.458-4.717)	1.081 (0.673-1.735)
	2017	10	-18.3 ± 0.6	14.1 ± 0.6	3.466 (2.925-4.107)	0.871 (0.727-1.044)
GET	2016	10	-19.3 ± 0.6	13.0 ± 1.0	4.816 (3.759-6.169)	0.991 (0.741-1.325)
	2017	10	-19.3 ± 0.8	12.8 ± 0.6	2.204 (1.615-3.009)	0.069 (0.046-0.103)
SAN	2017	10	-19.6 ± 0.7	12.6 ± 0.9	1.868 (1.356-2.575)	0.071 (0.040-0.124)
ULI	2016	10	-19.6 ± 0.5	12.3 ± 0.4	3.880 (3.137-4.798)	0.936 (0.628-1.393)
	2017	10	-19.3 ± 0.7	12.9 ± 0.6	2.401 (1.880-3.067)	0.103 (0.064-0.164)
JAI	2017	6	-19.4 ± 0.4	12.9 ± 0.7	3.752 (3.070-4.587)	1.072 (0.826-1.391)
BIA	2017	10	-18.6 ± 0.8	13.6 ± 0.7	2.608 (1.687-4.034)	0.229 (0.131-0.402)

Table 2 Ranking of the best models (ΔAICc<2), according to their small-sample size-corrected Akaike (AICc) values. Global model including all the possible factors and the null model corresponding to a constant model are also presented. Abbreviations: AICc, small sample size-corrected Akaike values; ΔAICc, difference in AICc values in relation to the first model; *df*, degrees of freedom; r^2, likelihood-ratio based R^2.

Models	AICc	ΔAICc	*df*	Deviance	r^2
log(MeHg)					
1. $\delta^{15}N + \delta^{13}C$ + Tarsus	97.0	0.00	7	82.05	0.45
2. $\delta^{15}N$ + Tarsus	97.9	0.90	6	85.20	0.43
3. $\delta^{13}C$ + Tarsus	99.0	1.97	6	86.27	0.43
Global	97.0	0.00	7	82.05	0.45
Null	146.4	49.4	4	138.1	0.13
log(HgT)					
1. $\delta^{15}N + \delta^{13}C$ + Tarsus	94.5	0.00	7	79.55	0.53
2. $\delta^{15}N$ + Tarsus	95.3	0.80	6	82.60	0.52
Global	94.5	0.00	7	79.55	0.53
Null	149.7	55.2	4	141.4	0.23
Prop.MeHg					
1. Tarsus	-320.4	0.00	5	-330.9	0.53
2. $\delta^{15}N + \delta^{13}C$ + Tarsus	-320.1	0.30	7	-335.1	0.54
3. $\delta^{15}N$ + Tarsus	-320.0	0.38	6	-332.7	0.53
4. $\delta^{15}N + \delta^{13}C$	-319.4	0.98	6	-332.1	0.53
Global	-320.1	0.30	7	-335.1	0.54
Null	-317.9	2.5	4	-326.2	0.51

Table 3 Beta-parameter estimates, SE and *P* value obtained after averaging the best-ranked models from Table 2. R.I.: relative variable importance in averaged model.

	log(MeHg)	log(HgT)	Prop.MeHg
$\delta^{15}N$	+0.18 ± 0.07 *P* = 0.016 R.I. = 0.81	+0.19 ± 0.07 *P* = 0.008 R.I. = 1.00	-0.02 ± 0.01 *P* = 0.146 R.I. = 0.70
$\delta^{13}C$	+0.19 ± 0.10 *P* = 0.058 R.I. = 0.68	+0.14 ± 0.08 *P* = 0.081 R.I. = 0.6	+0.02 ± 0.01 *P* = 0.099 R.I. = 0.45
Tarsus length	-0.02 ± 0.01 *P* = 0.003 R.I. = 1.00	-0.02 ± 0.01 *P* = 0.002 R.I. = 1.00	0.00 ± 0.00 *P* = 0.052 R.I. = 0.81

Fig 1 Map with the location of the ten colonies studied. Abbreviated names for the colonies are given (CAS: Castro, PUN: Punta Lucero, BIL: Billano, IZA: Izaro, LEK: Lekeitio, GET: Getaria, SAN: Santa Clara, ULI: Ulia, JAI: Jaizkibel and BIA: Biarritz).

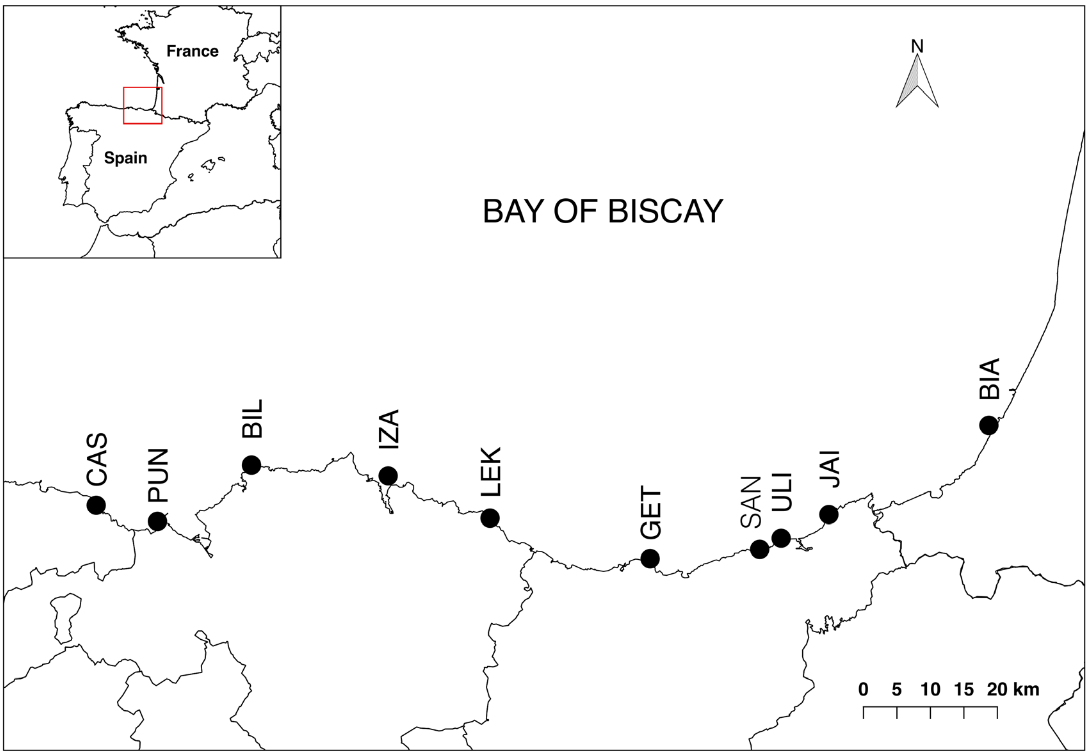


Fig. 2 Boxplots representing MeHg, Hg(II) and Prop.MeHg (proportion of MeHg over Hg) values in the ten sampling colonies. Colonies named as in Fig. 1. Boxplots represent: median, first and third quartile; whiskers extend 1.5 times the interquartile range; dots are extreme outliers.

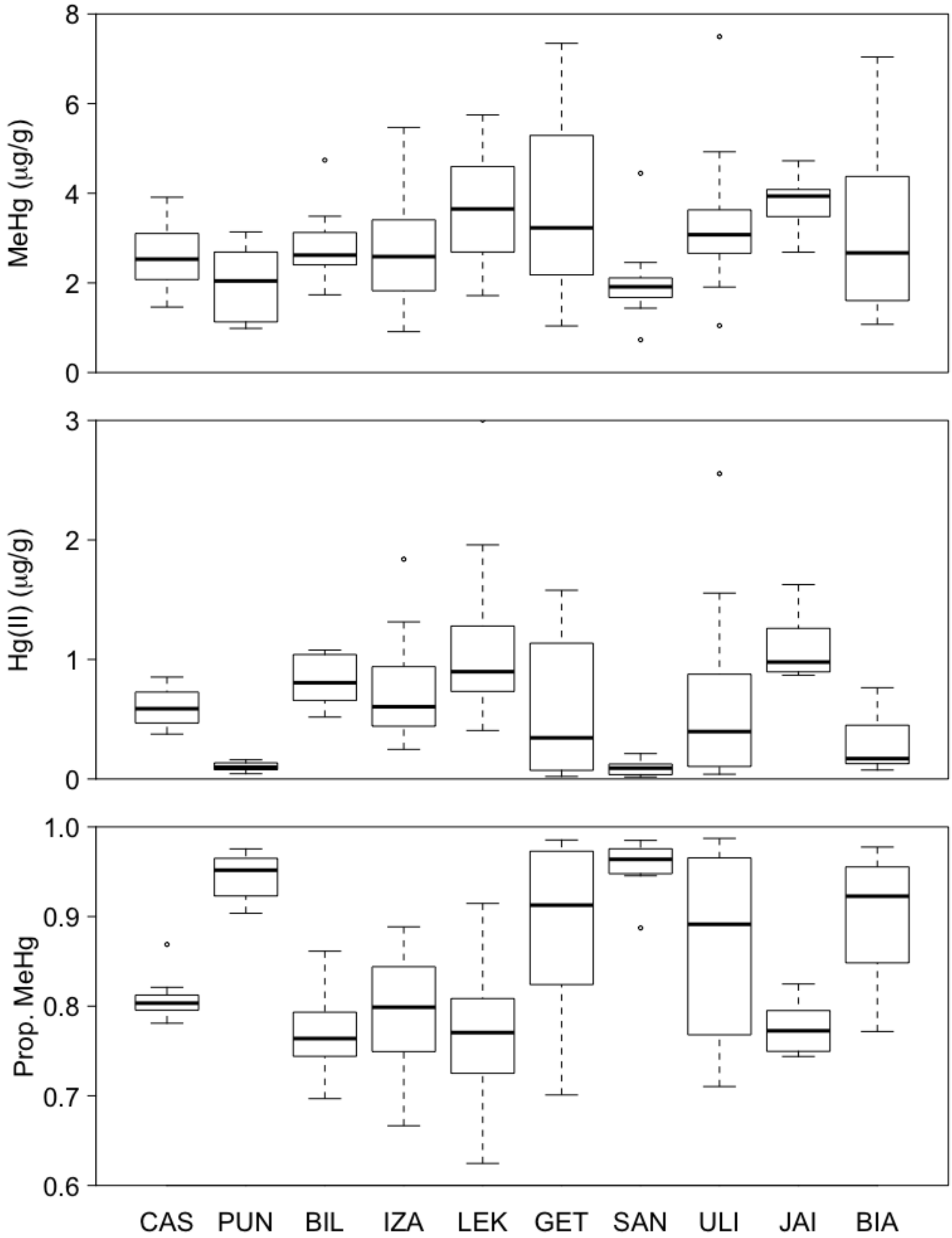
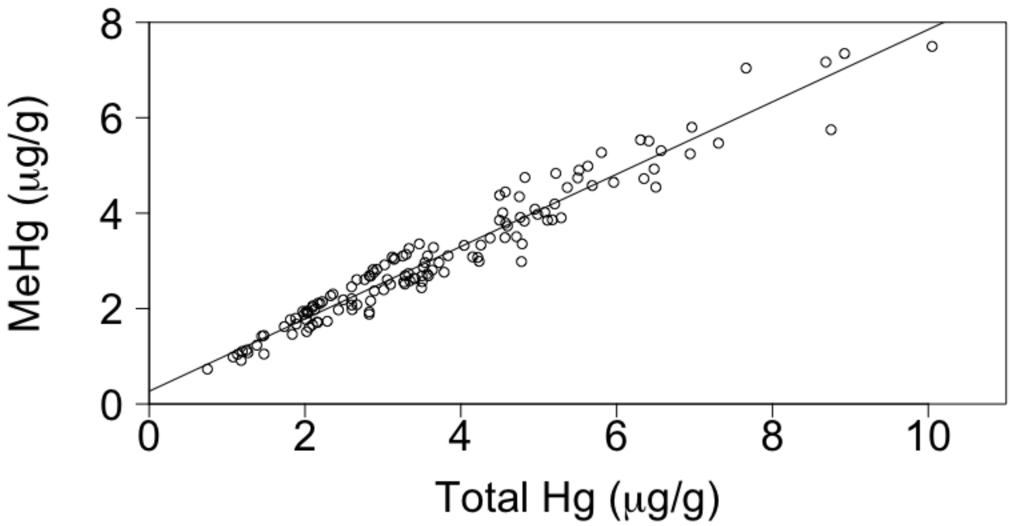


Fig. 3 Relationship between a) MeHg and HgT (Total Hg) and b) Prop.MeHg and HgT.

The line represents a linear relationship.

a)



b)

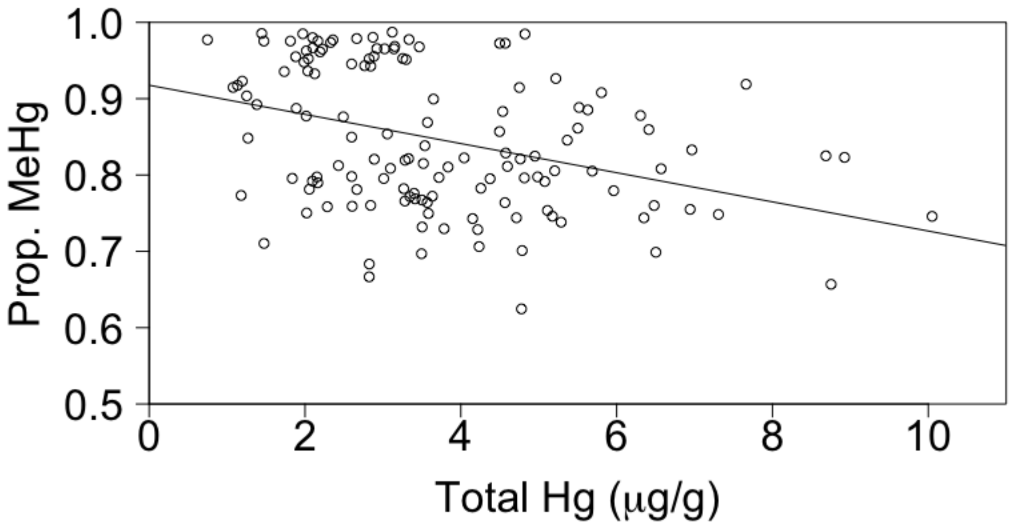


Fig. 4 $\delta^{13}C$ and $\delta^{15}N$ values for the ten colonies studied. Colonies named as in Fig. 1.