Seabird Tissues As Efficient Biomonitoring Tools for Hg Isotopic Investigations: Implications of Using Blood and Feathers from Chicks and Adults

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S *Supporting Information*

ABSTRACT: Blood and feathers are the two most targeted avian tissues for environmental biomonitoring studies, with mercury (Hg) concentration in blood and body feathers reflecting short and long-term Hg exposure, respectively. In this work, we investigated how Hg isotopic composition (e.g., δ^{202}Hg and Δ^{199}Hg) of blood and feathers from either seabird chicks (skuas, n = 40) or adults (penguins, n = 62) can accurately provide information on exposure to Hg in marine ecosystems. Our results indicate a strong correlation between blood and feather Hg isotopic values for skua chicks, with similar δ^{202}Hg and Δ^{199}Hg values in the two tissues (mean difference: −0.01 ± 0.25 ‰ and −0.05 ± 0.12 ‰, respectively). Since blood and body feathers of chicks integrate the same temporal window of Hg exposure, this

suggests that δ^{202}Hg and Δ^{199}Hg values can be directly compared without any correction factors within and between avian groups. Conversely, penguin adults show higher δ^{202}Hg and Δ^{199}Hg values in feathers than in blood (mean differences: 0.28 ± 0.19‰ and 0.25 ± 0.13‰), most likely due to tissue-specific Hg temporal integration. Since feathers integrate long-term (i.e., the intermoult period) Hg accumulation, whereas blood reflects short-term (i.e., seasonal) Hg exposure in adult birds, the two tissues provide complementary information on trophic ecology at different time scales.
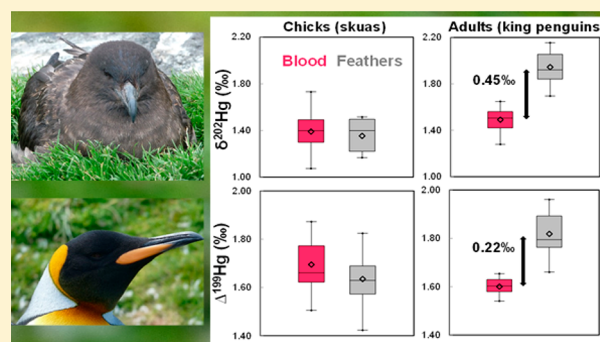
INTRODUCTION

Mercury (Hg) and more specifically methylmercury (MeHg) is a highly toxic environmental pollutant with severe risks for animal and human health.[1] The amount of Hg released into the environment has increased since preindustrial times as a consequence of human activities.[2,3] Due to its persistence and biomagnification in marine food webs, high levels of Hg have been reported in high trophic level predators.[4,5] For example, seabirds present elevated Hg concentrations in their tissues and have been reported as efficient bioindicators of the environmental pollution.[6] Since seabirds display contrasted foraging strategies and feed at different trophic levels, they are considered appropriate models to assess Hg contamination of the marine environment.

Hg exposure in birds is essentially attributed to dietary uptake (especially MeHg), which is then distributed by the bloodstream to internal organs and tissues.[7−9] Birds efficiently excrete Hg in feathers during plumage synthesis. Therefore, moulting is considered as the main detoxification route in most avian species.[10] Hg is sequestered in feathers by binding to

keratin molecules,[11] which impedes its reincorporation into internal tissues. Between 70 and 90% of the whole Hg body burden has been shown to be remobilised from internal tissues and excreted into the growing feathers,[9] mainly under its organic form (more than 90% of THg as MeHg).[12−14] In birds, blood and feathers (and also eggs) are the most frequently used tissues for biomonitoring studies because they do not involve lethal-sampling. Each tissue presents a specific Hg turnover rate, thus potentially providing complementary information at different temporal scales of Hg exposure. In adult birds, feathers, a metabolically inactive tissue, are representative of Hg incorporation during the intermoult period (one whole year in most seabirds). In contrast, blood is a metabolically active tissue representative of a shorter-term Hg intake than feathers, meaning a few weeks/months before sampling.[15] Therefore,

tissue-specific integration times must be considered for the selection of the most appropriate avian tissue depending on the scientific purposes.[16] In contrast, both sampled blood and simultaneously growing feathers of large chicks reflect recent Hg intake over a similar time-period, because chicks synthesize their first adult-like feathers at the end of the chick-rearing period.[16]

In the last decades, the measurement of Hg isotopic mass-dependent (MDF, δ^{202}Hg) and mass-independent fractionation (MIF, Δ^{199}Hg) has become a documented tool for identifying sources of Hg and biogeochemical processes within the different compartments of the environment.[17] Hg isotopes can vary as a result of fractionation during reactions and by mixing of isotopically distinct sources. Hg MDF can occur during all Hg specific transformation processes such as volatilization,[18] reduction,[19] bacterial methylation or demethylation reactions,[20−23] photochemical reactions[24] and metabolic processes .[25,26] A combination of these processes in the environment can induce similar or opposite MDF in different degrees of magnitude, so that processes and Hg reservoirs cannot so distinctly be recognized, especially when using complex bioindicators. In contrast, significant Hg MIF is induced exclusively during photochemical reactions and mainly concerns the two odd isotopes (199 and 201).[24,27] Hg MIF is not affected by biological processes[19,21,23] and its signature is thus assumed to be preserved throughout the food web.[25,28] Consequently, Hg MIF signatures in tissues of top predators allow investigating photochemical processes in the marine ecosystem prior to uptake in the food web.[24] Therefore, both Hg MDF and MIF provide complementary information and they are used as tracers for both Hg potential sources and transformation pathways in aquatic ecosystems.[29−33] Hg isotopic signatures have also been successfully used in ecotoxicological studies for a better understanding of detoxification and excretion processes and Hg metabolic responses, such as hepatic demethylation in marine mammals[26,34] or human exposure by excretion biomarkers such as urine[35] and hairs.[36] Concerning avian samples, two studies have revealed the efficiency of Hg isotopic analyses in seabird eggs for investigating factors controlling Hg cycling in aquatic ecosystems.[37,38]

Here, we present the first data on Hg isotopic composition (δ^{202}Hg and Δ^{199}Hg) of blood and body feathers of the same individual seabirds with the double objective of (i) investigating Hg isotopic relationships between the two tissues and (ii) evaluating their suitability as tracers of the fate of Hg in marine ecosystems. This study was performed on different seabird species from the Southern Ocean exhibiting contrasted ecological characteristics (i.e., food and feeding ecology) and breeding in distant sites (i.e., over a latitudinal gradient) in order to cover a wide range of tissue Hg concentrations.[39,40] We focused on two seabird models considered as representative of the local contamination, hence chicks (which were fed only with food from around the colony and reflect a relatively short period of exposure, 2−3 months) and penguins (which are more restricted to the colony area than migratory seabirds and reflect a longer period of exposure than chicks, one year). Since Hg integrated in chick blood and feathers corresponds to a similar time frame, we first focused on skua chicks, from four different populations, to explore inter-tissue isotopic relationships and the allocation of Hg following transport and potential fractionation from blood to feathers. Potential biological processes or transport among the two tissues were hypothe-

sized to induce Hg MDF, leading to differences in δ^{202}Hg values between blood and feathers. In contrast, Δ^{199}Hg values in blood and feathers of chicks were predicted to be similar, because Hg MIF is not induced by in vivo processes. In a second step, we investigated δ^{202}Hg and Δ^{199}Hg values in blood and feathers from adult penguins to evaluate the influence of specific Hg exposure temporal windows on Hg isotopic compositions of both tissues. We expect that seasonal changes in penguin food and feeding ecology can produce significant Hg isotopic variations among tissues. Penguins from six different species that breed from high-Antarctica to the subtropics were selected to confirm this hypothesis.

■ MATERIALS AND METHODS

Sites and Fieldwork. Sample collection was conducted during the austral summer 2011−2012 (from October to February) in four sites of the Terres Australes et Antarctiques Françaises, depending on seabird species: Pointe Géologie, Adélie Land (Antarctic Zone, 66°40′S, 140°10′E), Amsterdam Island (Subtropical Zone, 37°50′S, 77°31′E), Crozet Islands (Subantarctic Zone, 46°26′S, 51°45′E) and Kerguelen Islands (Subantarctic Zone, 49°21′S, 70°18′E) (SI Figure S1 and Table S1). Ten to 11 birds were randomly chosen in each group. Only one chick per skua nest was sampled to avoid pseudoreplication. Penguins were all adult breeding birds at the end of the reproductive cycle. Blood samples were collected from a wing vein, centrifuged, and red blood cells were kept frozen at −20 °C until analysis. A few body feathers were collected at random from the lower back of skuas and from the belly of penguins. Seabird chicks and adult penguins renew their entire plumage within a short time-period (i.e., 2−4 weeks), meaning that all body feathers grow almost simultaneously, thus showing very limited inter-feather variations in their Hg concentrations.[11,12]

Reference Materials, Sample Preparation, Total Hg, and Hg species Concentrations Analysis. Due to the absence of feather and bird blood certified reference materials (CRM) for Hg and MeHg concentrations, two internal reference samples were prepared with pooled samples collected from different individuals of king penguin (KP) from Crozet Islands: F-KP (feathers) and RBC-KP (red blood cells). The two reference samples were analyzed at each analytical session. For the validation of the results, human hair CRM (IAEA-086) was additionally analyzed due to its similar chemical composition to feathers (i.e., keratin). Feathers samples were cleaned, oven-dried and homogenized as detailed in a previous study.[41] Total Hg concentration (hereafter expressed as $\mu g\ g^{-1}$, dry weight) was also quantified by using an advanced Hg analyzer (AMA-254, Altec) thus allowing the intercomparison with Hg total concentrations obtained by Hg speciation analyses, that is, the sum of inorganic and organic Hg. For feather analyses, a matrix dependent calibration was performed with human hair reference material (NIES-13) as described elsewhere.[14] Blood sample analyses were performed as described in a previous study.[40] For Hg speciation analyses, feathers were prepared following a previously developed method.[14] Hg was extracted from blood samples (0.10−0.15 g) by alkaline microwave digestion with 5 mL of tetramethylammonium hydroxide (25% TMAH in H_2O, Sigma-Aldrich).[42] Details of the extraction method, analysis and quantification of Hg species are detailed elsewhere.[14]

Total Hg Isotopic Composition Analysis. Samples (0.05−0.10 g) were digested with 3 or 5 mL of HNO_3 acid

Table 1. Blood (Red Blood Cells) And Feather δ^{202}Hg and Δ^{199}Hg Values, Associated Statistics, And Isotopic Differences between Feathers and Blood of Skua Chicks[a]

species	location	n	blood δ^{202}Hg (‰) mean ± SD	feathers δ^{202}Hg (‰) mean ± SD	paired t-test (δ^{202}Hg values) t	p	paired δ^{202}Hg differences (‰) mean ± SD
Antarctic skua	Adélie Land	9	0.23 ± 0.13	0.00 ± 0.36	1.455	0.184	−0.16 ± 0.33
Subantarctic skua	Amsterdam	10	1.56 ± 0.08	1.51 ± 0.20	0.884	0.400	−0.06 ± 0.20
	Crozet	11	1.39 ± 0.18	1.35 ± 0.14	−3.209	0.512	−0.04 ± 0.18
	Kerguelen	10	1.04 ± 0.08	1.22 ± 0.19	−0.680	0.009	0.19 ± 0.18
	all populations	40			−0.333	0.741	−0.01 ± 0.25

species	location	n	blood Δ^{199}Hg (‰) mean ± SD	feathers Δ^{199}Hg (‰) mean ± SD	paired t- test (Δ^{199}Hg values) t	p	paired Δ^{199}Hg differences (‰) mean ± SD
Antarctic skua	Adélie Land	9	1.51 ± 0.08	1.46 ± 0.07	−1.096	0.305	−0.04 ± 0.11
Subantarctic skua	Amsterdam	10	1.76 ± 0.05	1.70 ± 0.05	**−2.440**	**0.037**	−0.06 ± 0.07
	Crozet	11	1.69 ± 0.11	1.64 ± 0.12	−1.454	0.180	−0.06 ± 0.17
	Kerguelen	10	1.61 ± 0.06	1.55 ± 0.12	−1.166	0.271	−0.05 ± 0.12
	all populations	40			**−2.745**	**0.008**	−0.05 ± 0.12

[a]Both tissues were sampled simultaneously in large chicks. Statistically significant results are marked in bold. Values are means ± SD.

(65%, INSTRA quality) after a predigestion step overnight at room temperature. Two different mineralization systems were successfully tested and used: High Pressure Asher (HPA) and Hotblock. HPA mineralization was performed at high conditions of pressure (130 bar) and temperature (temperature ramp: 80 °C to 120 °C (2 °C/min) to 300 °C (2.5 h) to 80 °C (1 h)). Hotblock mineralization was carried out in Savillex vessels at 75 °C during 8 h (6 h in HNO_3 and 2 h more after addition of $^1/_3$ of the total volume of H_2O_2 (30%, ULTREX quality)). Hg isotopic composition was determined using cold-vapor generator (CVG)-MC-ICPMS (Nu Instruments), as detailed previously.[30] Hg isotopic values were reported as delta notation, calculated relative to the bracketing standard NIST SRM-3133 reference material to allow interlaboratory comparisons, as described in the SI. NIST SRM-997 thallium standard solution was used for the instrumental mass-bias correction using the exponential law. Secondary standard NIST RM-8160 (previously UM-Almadén standard) was used for validation of the analytical session (SI Table S2).

Recoveries of extraction were verified for all samples by checking the signal intensity obtained on the MC-ICPMS for diluted extracts relative to NIST 3133 standard (with an approximate uncertainty of ±15%). Total Hg concentrations in the extract solution were compared to the concentrations found by AMA-254 analyses to assess method recovery. Average recoveries obtained were 98 ± 14% for feathers (n = 104) and 100 ± 2% for blood samples (n = 102). Accuracy of Hg isotopic analyses for keratin matrixes was evaluated with human hair material NIES-13, whose reference values are validated.[43] Hg isotopic results for soft-tissues (blood samples) were evaluated with validated reference values of Lake Michigan fish tissue NIST SRM 1947. Previously published Hg isotopic values for human hair IAEA-086 and tuna fish ERM-CE-464 materials were used for intercomparison.[43,44] Internal reference samples of feathers (F-KP) and avian blood (RBC-KP) were also measured. Repeatability was estimated by analyzing the same extract of each reference material over the long-term (during 2 weeks of analysis). Uncertainty for delta values was calculated using 2SD typical errors for each internal reference material (SI Table S2). Internal reproducibility was also assessed for numerous measurements of the two internal reference samples

of feathers (F-KP) and avian blood (RBC-KP) within 2−10 months of interval (SI Table S3).

Statistical Analyses. Statistical tests were performed using R 3.3.2 (RStudio).[45] Hg isotopic variations between blood and feathers from the same individuals of the different skua and penguin populations were assessed using paired t tests. Statistical differences between Hg MIF ratios for each seabird species were tested by nonparametrical test (Kruskal−Wallis with Conover-Iman test). Before these analyses, data were checked for normality of distribution and homogeneity of variances using Shapiro−Wilk and Breusch-Pagan tests, respectively. Statistically significant results were set at α = 0.05. Relationships between blood and feather Hg isotopic composition were tested using Pearson correlation rank tests.

RESULTS AND DISCUSSION

Information on Hg speciation in each tissue is essential for the interpretation of total Hg isotopic signatures. As expected, blood and feather samples analyzed in this study present a large proportion of MeHg (n = 102, 92 ± 6%, and 92 ± 3%, respectively). This is in agreement with previous reported results[14,46] and highlights the role of blood as a Hg transport tissue within the organism, including growing feathers.[15] Mean MeHg concentrations differed widely among skua chick populations for both blood (0.51−3.78 μg g^{-1}) and feathers (1.82−11.78 μg g^{-1}) and between adult penguin blood (0.43−1.95 μg g^{-1}) and feathers (0.35−3.90 μg g^{-1}) (SI Figures S2 and S3). Hg species concentrations for each seabird are included in SI Table S1.

Blood and feather Hg MDF (δ^{202}Hg) values followed the predicted theoretical MDF line[47,48] (calculation details in the SI). Hg odd-MIF were reported as Δ^{199}Hg values, i.e. the difference between measured δ^{199}Hg values and δ^{199}Hg values predicted by the theoretical MDF line. All blood and feather samples showed significant MIF of the odd isotopes (^{199}Hg and ^{201}Hg). Significant to very small MIF of even isotopes (Δ^{200}Hg) - a potential tracer of atmospherically sourced Hg[49−51]- was also observed (ranging from −0.14 to 0.17 ‰). No significant Δ^{200}Hg differences were found between blood and feather samples.

Skua Chicks: Identical Hg Isotopic Composition in Blood and Feathers. Chick blood δ^{202}Hg values range from
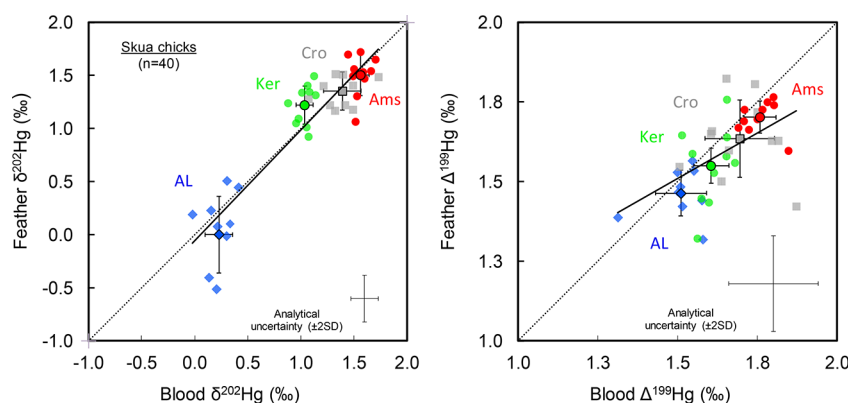
Figure 1. Feather versus blood (red blood cells) δ^{202}Hg and Δ^{199}Hg values in skua chicks (individual values and mean population values). Regression equations for δ^{202}Hg blood-feather and Δ^{199}Hg blood-feather values are $y = 1.12x - 0.17$ ($R^2 = 0.81$, $p < 0.0001$), and $y = 0.63x - 0.56$ ($R^2 = 0.33$, $p < 0.0001$), respectively. Abbreviations for the different populations are AL (Adélie Land), Ker (Kerguelen), Cro (Crozet) and Ams (Amsterdam).

Table 2. Blood (Red Blood Cells) And Feather δ^{202}Hg and Δ^{199}Hg Values, Associated Statistics, And Isotopic Differences between Feathers and Blood of Adult Penguins[a]

species	location	n	blood δ^{202}Hg (‰)	feathers δ^{202}Hg (‰)	paired t-test (δ^{202}Hg values)		paired δ^{202}Hg differences (‰)
			mean ± SD	mean ± SD	T	P	mean ± SD
Adélie penguin	Adélie Land	10	0.56 ± 0.20	0.67 ± 0.13	2.147	0.060	0.11 ± 0.16
Northern rockhopper penguin	Amsterdam	10	2.16 ± 0.14	2.42 ± 0.13	5.601	<0.0001	0.27 ± 0.15
Gentoo penguin	Crozet	11	1.45 ± 0.12	1.43 ± 0.10	−0.422	0.682	−0.02 ± 0.12
King penguin	Crozet	11	1.49 ± 0.11	1.94 ± 0.15	7.909	<0.0001	0.45 ± 0.19
Macaroni penguin	Crozet	10	1.66 ± 0.11	2.01 ± 0.15	5.453	<0.0001	0.35 ± 0.20
Eastern rockhopper penguin	Crozet	10	1.93 ± 0.18	2.31 ± 0.12	8.379	<0.0001	0.38 ± 0.14
	all populations	62			−8.795	<0.0001	0.26 ± 0.23

| species	location	n	blood Δ^{199}Hg (‰)	feathers Δ^{199}Hg (‰)	paired t-test (Δ^{199}Hg values)		paired Δ^{199}Hg differences (‰)
			mean ± SD	mean ± SD	T	P	mean ± SD
Adélie penguin	Adélie Land	10	1.54 ± 0.11	1.80 ± 0.14	**8.330**	**<0.0001**	0.26 ± 0.10
Northern rockhopper penguin	Amsterdam	10	1.89 ± 0.12	2.22 ± 0.10	**59.061**	**<0.0001**	0.33 ± 0.08
Gentoo penguin	Crozet	11	1.41 ± 0.06	1.51 ± 0.12	**2.902**	**0.016**	0.10 ± 0.12
King penguin	Crozet	11	1.60 ± 0.04	1.82 ± 0.09	**7.215**	**<0.0001**	0.22 ± 0.10
Macaroni penguin	Crozet	10	1.54 ± 0.06	1.86 ± 0.08	**11.410**	**<0.0001**	0.31 ± 0.09
Eastern rockhopper penguin	Crozet	10	1.77 ± 0.13	2.09 ± 0.09	**−7.290**	**<0.0001**	0.32 ± 0.14
	all populations	62			**−15.390**	**<0.0001**	0.25 ± 0.13

[a]Both tissues were sampled simultaneously in adults. Statistically significant results are marked in bold. Values are means ± SD.

0.23 ± 0.13 to 1.56 ± 0.08‰. Likewise, feather δ^{202}Hg values vary widely, from 0.00 ± 0.36 to 1.51 ± 0.20‰ (Table 1). Low variability is found in Δ^{199}Hg between the four sites, with blood values ranging from 1.51 ± 0.08 to 1.70 ± 0.05‰ and feather values varying from 1.46 ± 0.07 to 1.76 ± 0.05‰ (Table 1). The range of δ^{202}Hg and Δ^{199}Hg values obtained for the different skua populations are within the range of previous measurement performed on marine and pelagic organisms such as seabird eggs[37,38] and pelagic fish.[33,52] Skua chick blood samples display an overall Δ^{199}Hg/Δ^{201}Hg slope of 1.14 ± 0.07 (Pearson correlation, $R^2 = 0.86$, $p < 0.0001$), while skua chick feathers present a Δ^{199}Hg/Δ^{201}Hg slope of 1.13 ± 0.05 (Pearson correlation, $R^2 = 0.57$, $p < 0.0001$). These slope values fall within the range of values of other marine organisms, including seabirds,[33,37,52] and they were linked to photo-demethylation of MeHg (i.e., the magnetic isotope effect).[24] No statistical differences of blood and feathers Δ^{199}Hg/Δ^{201}Hg were found among the four skua populations. No statistical tissue-specific differences were observed between blood and

feather Δ^{199}Hg/Δ^{201}Hg ratios, except for Adélie Land skuas (Kruskal−Wallis, $H = 3.841$, $p = 0.031$). Such difference for Adélie Land skuas can be explained by the low odd-MIF values and a larger dispersion of the isotopic data obtained for their feather samples.

Individual paired differences of δ^{202}Hg and Δ^{199}Hg (feathers minus blood) amounted to −0.01 ± 0.25‰ and −0.05 ± 0.12‰, respectively, when pooling all the individuals ($n = 40$). Feather and blood δ^{202}Hg values were not overall statistically different in skua chicks except for Kerguelen individuals (Table 1). In the same way, mean Δ^{199}Hg paired differences observed for each skua chick population were not statistically significant, except for Amsterdam individuals (Table 1).

Linear regressions between blood and feather Hg isotopic values were determined for all the individuals (Figure 1). They showed a high and positive correlation for δ^{202}Hg values (Pearson correlation, $R^2 = 0.91$, $p < 0.0001$, $n = 40$). Δ^{199}Hg values were also significantly correlated but to a lesser extent (Pearson correlation, $R^2 = 0.53$, $p < 0.0001$, $n = 40$). Regression
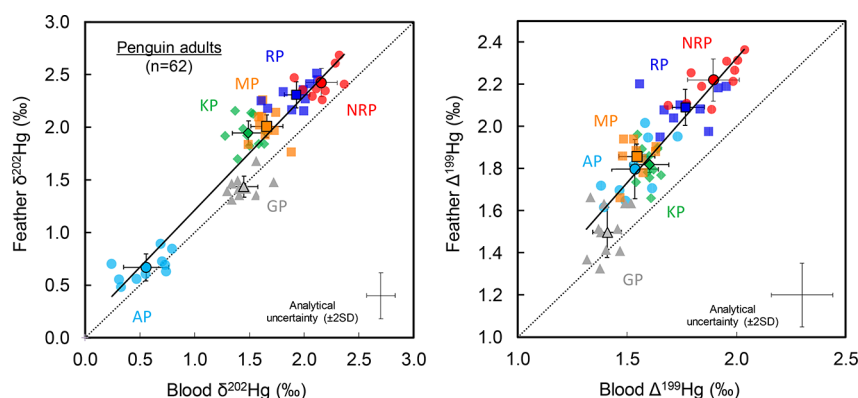
Figure 2. Feather versus blood (red blood cells) $\delta^{202}Hg$ and $\Delta^{199}Hg$ values in adult penguins (individual values and mean population values). Regression equations for $\delta^{202}Hg$ blood-feather and $\Delta^{199}Hg$ blood-feather values are $y = 1.08x + 0.13$ ($R^2 = 0.86$, $p < 0.0001$) and $y = 1.18x - 0.04$ ($R^2 = 0.75$, $p < 0.0001$), respectively. Abbreviations for the different populations are AP (Adélie penguin), GP (gentoo penguin), KP (king penguin), MP (macaroni penguin), RP (eastern rockhopper penguin), and NRP (northern rockhopper penguin).

slopes were not statistically different from slope 1:1 for $\delta^{202}Hg$ ($t = -12.68$, $p < 0.0001$) and $\Delta^{199}Hg$ values ($t = -6.54$, $p < 0.0001$).

A major result of this study is that skua chicks exhibit identical $\delta^{202}Hg$ and $\Delta^{199}Hg$ values in blood and feathers, including similar $\Delta^{199}Hg/\Delta^{201}Hg$ slopes. This strongly suggests that no metabolic processes influence $\delta^{202}Hg$ (and $\Delta^{199}Hg$) during feather growth. Consequently, blood and feathers can be directly compared without applying correction factors, as it is the case for $\delta^{13}C$ and $\delta^{15}N$ values.[53] This finding has important practical consequences for biogeochemical studies using birds as bioindicators because either feathers or blood can be easily and non-destructively sampled in the field. In other words, the lack of significant differences among the two tissues suggests that blood and feather $\delta^{202}Hg$ and $\Delta^{199}Hg$ values can be compared confidently within and between various avian data sets that include chicks.

Penguin Adults: Differences in Hg Isotopic Composition between Blood and Feathers. Penguin $\delta^{202}Hg$ values ranged from 0.56 ± 0.20 to $2.16 \pm 0.14‰$ and from 0.67 ± 0.13 to $2.42 \pm 0.13‰$ for blood and feathers, respectively (Table 2). Penguin blood $\Delta^{199}Hg$ values vary from 1.41 ± 0.06 to $1.89 \pm 0.12‰$, whereas penguin feathers exhibit slightly higher $\Delta^{199}Hg$ values that ranged from 1.51 ± 0.12 to $2.22 \pm 0.10‰$ (Table 2). Again, the results obtained for Hg isotopic compositions (both $\delta^{202}Hg$ and $\Delta^{199}Hg$ values) are close to those determined in previous studies performed on marine biological organisms.[33,37,38] Significantly different Hg isotopic values were found among the two tissues. Feathers showed greater $\delta^{202}Hg$ and $\Delta^{199}Hg$ values than blood in all species but the gentoo penguin. Respective mean paired differences for $\delta^{202}Hg$ and $\Delta^{199}Hg$ were 0.26 ± 0.23 and $0.25 \pm 0.13 ‰$, respectively (all individuals, $n = 62$). However, gentoo penguins show much lower mean paired differences, that is, $-0.02 \pm 0.12‰$ ($\delta^{202}Hg$) and $0.10 \pm 0.12‰$ ($\Delta^{199}Hg$), compared to the other penguin species. Paired differences of pooled samples were statistically significant for both $\delta^{202}Hg$ and $\Delta^{199}Hg$ values (Table 2). Penguin blood and feather samples displayed $\Delta^{199}Hg/\Delta^{201}Hg$ slopes of 1.17 ± 0.06 ($R^2 = 0.85$, $p < 0.0001$) and 1.09 ± 0.11 ($R^2 = 0.95$, $p < 0.0001$), respectively. The values are within the range of slopes observed for marine organisms[33,37,52] and photochemically induced odd-MIF.[24] No statistically different feather $\Delta^{199}Hg/\Delta^{201}Hg$ ratios were found between penguin species. For blood, no statistical $\Delta^{199}Hg/$

$\Delta^{201}Hg$ ratios differences were found between penguins, except for northern rockhopper penguins ($H = 17.38$, $p = 0.004$).

There was a high positive correlation among tissues for both $\delta^{202}Hg$ (Pearson correlation, $R^2=0.93$ $p < 0.0001$, $n = 62$) and $\Delta^{199}Hg$ values (Pearson correlation, $R^2 = 0.87$, $p < 0.0001$, $n = 62$) (Figure 2). Regression slopes were not statistically different from 1:1, neither for $\delta^{202}Hg$ ($t = -22.01$, $p < 0.0001$) nor for $\Delta^{199}Hg$ values ($t = -14.41$, $p < 0.0001$).

Both non-exclusive intrinsic and extrinsic factors may account for the tissue-specific isotopic differences in adult penguins. Intrinsic factors include different bioaccumulation or metabolic pathways that might induce MDF between internal tissues.[25,26,54] Internal processes such as inter-tissue transport, metabolic transformations or Hg complexation with proteins could hypothetically induce MDF between blood and feathers. Depending on populations, adult penguins showed either no significant (gentoo penguins) or significant isotopic differences between blood and feathers. Such interspecies variations suggest that internal processes from blood to the growing feathers were not responsible of Hg isotope MDF ($\delta^{202}Hg$) differences between the two tissues, which is also in agreement with identical blood and feather $\delta^{202}Hg$ values in skua chicks. Hg MIF is preserved during in vivo processes.[26,30] Hence, $\Delta^{199}Hg$ differences between blood and feathers of adult penguins are not linked to metabolic processes, but, instead, to external environmental factors that are likely to also drive tissue-specific $\delta^{202}Hg$ differences.

Among potential external factors, both temporal and geographical mismatch of Hg integration between blood and feathers could induce Hg isotopic variations for both $\delta^{202}Hg$ and $\Delta^{199}Hg$ values between adult penguin tissues. Hg odd-MIF magnitude (and in less extent MDF) is known to vary significantly as a function of photochemical processes, therefore Hg odd-MIF extent should be more important during summertime when solar radiation is greater. Since adult seabird blood reflects Hg integrated during summer whereas feathers represent Hg accumulated over one year, blood should present higher $\Delta^{199}Hg$ values than feathers for the same foraging habitat. Unexpectedly, higher $\Delta^{199}Hg$ values were found in adult feathers, thus suggesting that odd-MIF shifts between tissues are related to seasonal changes in the foraging habitat. As already evidenced in oceanic ecosystems, Hg odd-MIF is highly dependent on the specific environmental conditions of light incidence both at spatial[33] and depth scales.[52] Adult

seabirds foraging at more opened oceanic waters, regions distant from the colonies and/or at lower depths of the water column during their interbreeding period may then present higher Δ^{199}Hg values in their feathers than in blood as a consequence of higher light penetration conditions. The very homogeneous values obtained for Δ^{199}Hg/Δ^{201}Hg slope do not help here to differentiate any specific signature for the bioaccumulated MeHg, and only support that most of the MeHg present in penguin tissues originate from pelagic environments. Unlike other penguins, gentoo penguins showed no tissue-related isotopic differences, a characteristic that can be explained by their nonmigratory behavior. Since they stay all year long in coastal waters near their colonies,[55] both blood and feathers reflect Hg exposure from the same habitat over summer and a full year, respectively, thus reducing inter-tissue Hg isotopic differences. For the other five penguin populations, the Hg isotopic differences between blood and feathers are likely the consequence of birds dispersing over relatively large areas during the interbreeding period.[56−58] Blood isotopic values correspond to the summer breeding period, but feathers also integrate the premoulting trip and the interbreeding migration far way the breeding grounds where birds are likely to feed on different prey with different isotopic δ^{202}Hg and Δ^{199}Hg values.

Tissue-Specific Integration Time: Implication for Hg Isotopes Biomonitoring. Differences of Hg isotopic signatures between adult blood and feathers reflect changes in their feeding ecology throughout the year due to different diet and Hg exposure integration times among both tissues. Adult feathers sequestrate Hg acquired between two moults (approximatively 12 months for penguins), thus presenting a much longer Hg accumulation interval compared to blood. Generally, adult feathers present higher Hg isotopic values than blood (both δ^{202}Hg and Δ^{199}Hg) suggesting their Hg integration from additional sources in which MeHg is exposed to higher photochemical processes. Feather Hg isotopic information integrates the various foraging habitats over the birds' annual cycle, thus providing integrated information on the whole-year Hg exposure. Thus, a correct interpretation of feather Hg isotopic composition implies a good knowledge of seabird trophic ecology all year long. Unlike feathers, adult blood isotopic information concerns only Hg exposure during the breeding period (recent exposure). In contrast, seabird chicks start synthetizing their first feathers in the second part of their rearing-period and therefore both blood and feathers represent similar periods of Hg dietary intake at the end of the breeding period.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b00422.

> Supplementary data, figures, tables, methodology, and discussion (PDF)

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REFERENCES

(1) Tan, S. W.; Meiller, J. C.; Mahaffey, K. R. The endocrine effects of mercury in humans and wildlife. *Crit. Rev. Toxicol.* **2009**, *39* (3), 228−269.

(2) Mason, R. P.; Choi, A. L.; Fitzgerald, W. F.; Hammerschmidt, C. R.; Lamborg, C. H.; Soerensen, A. L.; Sunderland, E. M. Mercury biogeochemical cycling in the ocean and policy implications. *Environ. Res.* **2012**, *119*, 101−117.

(3) Fitzgerald, W. F.; Lamborg, C. H.; Hammerschmidt, C. R. Marine biogeochemical cycling of mercury. *Chem. Rev.* **2007**, *107* (2), 641−662.

(4) Bargagli, R.; Monaci, F.; Sanchez-Fernandez, C.; Cateni, D. Biomagnification of mercury in an Antarctic marine coastal food web. *Mar. Ecol.: Prog. Ser.* **1998**, *169*, 65−76.

(5) Hammerschmidt, C. R.; Fitzgerald, W. F. Bioaccumulation and trophic transfer of methylmercury in Long Island Sound. *Arch. Environ. Contam. Toxicol.* **2006**, *51* (3), 416−424.

(6) Burger, J.; Gochfeld, M. Marine birds as sentinels of environmental pollution. *EcoHealth J. Consort.* **2004**, *1* (3), 263−274.

(7) Thompson, D. R.; Furness, R. W. The chemical form of mercury stored in South Atlantic seabirds. *Environ. Pollut.* **1989**, *60*, 305−317.

(8) Braune, B. M. Comparison of total mercury levels in relation to diet and molt for nine species of marine birds. *Arch. Environ. Contam. Toxicol.* **1987**, *16*, 217−224.

(9) Honda, K.; Nasu, T.; Tatsukawa, R. Seasonal changes in mercury accumulation in the black-eared kite, *Milvus migrans lineatus. Environ. Pollut., Ser. A* **1986**, *42*, 325−334.

(10) Furness, R. W.; Muirhead, S. J.; Woodburn, M. Using bird feathers to measure mercury in the environment: Relationships between mercury content and moult. *Mar. Pollut. Bull.* **1986**, *17* (1), 27−30.

(11) Appelquist, H.; Asbirk, S.; Drabaek, I. Mercury monitoring: mercury stability in bird feathers. *Mar. Pollut. Bull.* **1984**, *15* (I), 22−24.

(12) Thompson, D. R.; Furness, R. W. Differences in the chemical form of mercury stored in South Atlantic seabirds. *Environ. Pollut.* **1989**, *60*, 305−317.

(13) Thompson, D. R.; Stewart, F. M.; Furness, R. W. Using seabirds to monitor mercury in marine environments. The validity of conversion ratios for tissue comparisons. *Mar. Pollut. Bull.* **1990**, *21* (7), 339−342.

(14) Renedo, M.; Bustamante, P.; Tessier, E.; Pedrero, Z.; Cherel, Y.; Amouroux, D. Assessment of mercury speciation in feathers using species-specific isotope dilution analysis. *Talanta* **2017**, *174*, 100−110.

(15) Bearhop, S.; Ruxton, G. D.; Furness, R. W. Dynamics of mercury in blood and feathers of greet skuas. *Environ. Toxicol. Chem.* **2000**, *19* (6), 1638−1643.

(16) Carravieri, A.; Bustamante, P.; Churlaud, C.; Fromant, A.; Cherel, Y. Moulting patterns drive within-individual variations of stable

isotopes and mercury in seabird body feathers: implications for monitoring of the marine environment. *Mar. Biol.* **2014**, *161*, 963–968.

(17) Blum, J. D.; Sherman, L. S.; Johnson, M. W. Mercury isotopes in Earth and environmental sciences. *Annu. Rev. Earth Planet. Sci.* **2014**, *42*, 249–269.

(18) Zheng, W.; Foucher, D.; Hintelmann, H. Mercury isotope fractionation during volatilization of Hg(0) from solution into the gas phase. *J. Anal. At. Spectrom.* **2007**, *22* (9), 1097.

(19) Kritee, K.; Blum, J. D.; Johnson, M. W.; Bergquist, B. A.; Barkay, T. Mercury stable isotope fractionation during reduction of Hg(II) to Hg(0) by mercury resistant microorganisms. *Environ. Sci. Technol.* **2007**, *41* (6), 1889–1895.

(20) Rodriguez Gonzalez, P.; Epov, V. N.; Bridou, R.; Tessier, E.; Guyoneaud, R.; Monperrus, M.; Amouroux, D. Species-specific stable isotope fractionation of mercury during Hg (II) methylation by an anaerobic bacteria (Desulfobulbus propionicus) under dark conditions. *Environ. Sci. Technol.* **2009**, *43* (24), 9183–9188.

(21) Kritee, K.; Barkay, T.; Blum, J. D. Mass dependent stable isotope fractionation of mercury during mer mediated microbial degradation of monomethylmercury. *Geochim. Cosmochim. Acta* **2009**, *73* (5), 1285–1296.

(22) Perrot, V.; Jimenez-Moreno, M.; Berail, S.; Epov, V. N.; Monperrus, M.; Amouroux, D. Successive methylation and demethylation of methylated mercury species (MeHg and DMeHg) induce mass dependent fractionation of mercury isotopes. *Chem. Geol.* **2013**, *355*, 153–162.

(23) Perrot, V.; Bridou, R.; Pedrero, Z.; Guyoneaud, R.; Monperrus, M.; Amouroux, D. Identical Hg isotope mass dependent fractionation signature during methylation by sulfate-reducing bacteria in sulfate and sulfate-free environment. *Environ. Sci. Technol.* **2015**, *49* (3), 1365–1373.

(24) Bergquist, B. A.; Blum, J. D. Mass-dependent and -independent fractionation of Hg isotopes by photoreduction in aquatic systems. *Science* **2007**, *318* (5849), 417–420.

(25) Feng, C.; Pedrero, Z.; Gentès, S.; Barre, J.; Renedo, M.; Tessier, E.; Berail, S.; Maury-Brachet, R.; Mesmer-Dudons, N.; Baudrimont, M.; et al. Specific pathways of dietary methylmercury and inorganic mercury determined by mercury speciation and isotopic composition in zebrafish (Danio rerio). *Environ. Sci. Technol.* **2015**, *49* (21), 12984–12993.

(26) Perrot, V.; Masbou, J.; Pastukhov, M. V.; Epov, V. N.; Point, D.; Bérail, S.; Becker, P. R.; Sonke, J. E.; Amouroux, D. Natural Hg isotopic composition of different Hg compounds in mammal tissues as a proxy for in vivo breakdown of toxic methylmercury. *Metallomics* **2016**, *8* (2), 170–178.

(27) Zheng, W.; Hintelmann, H. Isotope fractionation of mercury during its photochemical reduction by low-molecular-weight organic compounds. *J. Phys. Chem. A* **2010**, *114*, 4246–4253.

(28) Kwon, S. Y.; Blum, J. D.; Carvan, M. J.; Basu, N.; Head, J. A.; Madenjian, C. P.; David, S. R. Absence of fractionation of mercury isotopes during trophic transfer of methylmercury to freshwater fish in captivity. *Environ. Sci. Technol.* **2012**, *46* (14), 7527–7534.

(29) Li, M.; Schartup, A. T.; Valberg, A. P.; Ewald, J. D.; David, P.; Yin, R.; Balcom, P. H.; Sunderland, E. M. Environmental origins of methylmercury accumulated in subarctic estuarine fish indicated by mercury stable isotopes. *Environ. Sci. Technol.* **2016**, *50*, 11559–15568.

(30) Perrot, V.; Pastukhov, M. V.; Epov, V. N.; Husted, S.; Donard, O. F. X.; Amouroux, D. Higher mass-independent isotope fractionation of methylmercury in the pelagic food web of Lake Baikal (Russia). *Environ. Sci. Technol.* **2012**, *46* (11), 5902–5911.

(31) Jackson, T. A.; Whittle, D. M.; Evans, M. S.; Muir, D. C. G. Evidence for mass-independent and mass-dependent fractionation of the stable isotopes of mercury by natural processes in aquatic ecosystems. *Appl. Geochem.* **2008**, *23* (3), 547–571.

(32) Gehrke, G. E.; Blum, J. D.; Marvin-DiPasquale, M. Sources of mercury to San Francisco Bay surface sediment as revealed by mercury stable isotopes. *Geochim. Cosmochim. Acta* **2011**, *75* (3), 691–705.

(33) Senn, D. B.; Chesney, E. J.; Blum, J. D.; Bank, M. S.; Maage, A.; Shine, J. P. Stable isotope (N, C, Hg) study of methylmercury sources and trophic transfer in the northern Gulf of Mexico. *Environ. Sci. Technol.* **2010**, *44* (5), 1630–1637.

(34) Masbou, J.; Point, D.; Sonke, J. E.; Frappart, F.; Perrot, V.; Amouroux, D.; Richard, P.; Becker, P. R. Hg stable isotope time trend in ringed seals registers decreasing sea ice cover in the Alaskan Arctic. *Environ. Sci. Technol.* **2015**, *49* (15), 8977–8985.

(35) Sherman, L. S.; Blum, J. D.; Franzblau, A.; Basu, N. New insight into biomarkers of human mercury exposure using naturally occurring mercury stable isotopes. *Environ. Sci. Technol.* **2013**, *47*, 3403–3409.

(36) Laffont, L.; Sonke, J. E.; Maurice, L.; Hintelmann, H.; Pouilly, M.; Sanchez Bacarreza, Y.; Perez, T.; Behra, P. Anomalous mercury isotopic compositions of fish and human hair in the Bolivian Amazon. *Environ. Sci. Technol.* **2009**, *43*, 8985–8990.

(37) Point, D.; Sonke, J. E.; Day, R. D.; Roseneau, D. G.; Hobson, K. A.; Pol, S. S. Vander; Moors, A. J.; Pugh, R. S.; Donard, O. F. X.; Becker, P. R. Methylmercury photodegradation influenced by sea-ice cover in Arctic marine ecosystems. *Nat. Geosci.* **2011**, *4* (1), 1–7.

(38) Day, R. D.; Roseneau, D. G.; Berail, S.; Hobson, K. a.; Donard, O. F. X.; Vander Pol, S. S.; Pugh, R. S.; Moors, A. J.; Long, S. E.; Becker, P. R. Mercury stable isotopes in seabird eggs reflect a gradient from terrestrial geogenic to oceanic mercury reservoirs. *Environ. Sci. Technol.* **2012**, *46* (10), 5327–5335.

(39) Carravieri, A.; Cherel, Y.; Jaeger, A.; Churlaud, C.; Bustamante, P. Penguins as bioindicators of mercury contamination in the southern Indian Ocean: geographical and temporal trends. *Environ. Pollut.* **2016**, *213*, 195–205.

(40) Carravieri, A.; Cherel, Y.; Brault-favrou, M.; Churlaud, C.; Peluhet, L.; Labadie, P.; Bustamante, P. From Antarctica to the subtropics : Contrasted geographical concentrations of selenium, mercury, and persistent organic pollutants in skua chicks (Catharacta spp.). *Environ. Pollut.* **2017**, *228*, 464–473.

(41) Carravieri, A.; Bustamante, P.; Churlaud, C.; Cherel, Y. Penguins as bioindicators of mercury contamination in the Southern Ocean: birds from the Kerguelen Islands as a case study. *Sci. Total Environ.* **2013**, *454–455*, 141–148.

(42) Rodrigues, J. L.; Rodriguez Alvarez, C.; Rodriguez Farinas, N.; Berzas Nevado, J. J.; Barbosa, F. J.; Rodriguez Martin-Doimeadios, R. C. Mercury speciation in whole blood by gas chromatography coupled to ICP-MS with a fast microwave-assisted sample preparation procedure. *J. Anal. At. Spectrom.* **2011**, *26*, 436–442.

(43) Yamakawa, A.; Takeuchi, A.; Shibata, Y.; Berail, S.; Donard, O. F. X. Determination of Hg isotopic compositions in certified reference material NIES No. 13 Human Hair by cold vapor generation multicollector inductively coupled plasma mass spectrometry. *Accredit. Qual. Assur.* **2016**, No. 13.19710.1007/s00769-016-1196-x

(44) Li, M.; Sherman, L. S.; Blum, J. D.; Grandjean, P.; Weihe, P.; Sunderland, E. M.; Shine, J. P. Assessing sources of human methylmercury exposure using stable mercury isotopes. *Environ. Sci. Technol.* **2014**, *48*, 8800–8806.

(45) R Core Team. *A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2016.

(46) Thompson, D. R.; Furness, R. W. Comparison of the levels of total and organic mercury in seabird feathers. *Mar. Pollut. Bull.* **1989**, *20* (11), 577–579.

(47) Young, E. D.; Galy, A.; Nagahara, H. Kinetic and equilibrium mass-dependant isotope fractionation laws in nature and their geochemical and cosmochemical significance. *Geochim. Cosmochim. Acta* **2002**, *66* (6), 1095–1104.

(48) Blum, J. D.; Bergquist, B. a. Reporting of variations in the natural isotopic composition of mercury. *Anal. Bioanal. Chem.* **2007**, *388*, 353–359.

(49) Chen, J.; Hintelmann, H.; Feng, X.; Dimock, B. Unusual fractionation of both odd and even mercury isotopes in precipitation from Peterborough, ON, Canada. *Geochim. Cosmochim. Acta* **2012**, *90*, 33–46.

(50) Sherman, L. S.; Blum, J. D.; Douglas, T. A.; Steffen, A. Frost flowers growing in the Arctic ocean-atmosphere-sea ice-snow interface: 2. Mercury exchange between the atmosphere, snow, and frost flowers. *J. Geophys. Res. Atmos.* **2012**, *117* (3), 1−10.

(51) Wang, Z.; Chen, J.; Feng, X.; Hintelmann, H.; Yuan, S.; Cai, H.; Huang, Q.; Wang, S.; Wang, F. Mass-dependent and mass-independent fractionation of mercury isotopes in precipitation from Guiyang, SW China. *C. R. Geosci.* **2015**, *347* (7−8), 358−367.

(52) Blum, J. D.; Popp, B. N.; Drazen, J. C.; Anela Choy, C.; Johnson, M. W. Methylmercury production below the mixed layer in the North Pacific Ocean. *Nat. Geosci.* **2013**, *6* (10), 879−884.

(53) Cherel, Y.; Jaquemet, S.; Maglio, A.; Jaeger, A. Differences in δ13C and δ15N values between feathers and blood of seabird chicks: Implications for non-invasive isotopic investigations. *Mar. Biol.* **2014**, *161* (1), 229−237.

(54) Cransveld, A. A. E.; Amouroux, D.; Tessier, E.; Koutrakis, E.; Ozturk, A. A.; Bettoso, N.; Mieiro, C. L.; Berail, S.; Barre, J. P. G.; Sturaro, N.; et al. Mercury stable isotopes discriminate different populations of European seabass and trace potential Hg sources around Europe. *Environ. Sci. Technol.* **2017**, *51* (21), 12219−12228.

(55) Lescroël, A.; Ridoux, V.; Bost, C. A. Spatial and temporal variation in the diet of the gentoo penguin (Pygoscelis papua) at Kerguelen Islands. *Polar Biol.* **2004**, *27* (4), 206−216.

(56) Cherel, Y.; Hobson, K. A.; Guinet, C.; Vanpe, C. Stable isotopes document seasonal changes in trophic niches and winter foraging individual specialization in diving predators from the Southern Ocean. *J. Anim. Ecol.* **2007**, *76* (4), 826−836.

(57) Bost, C. A.; Cotté, C.; Terray, P.; Barbraud, C.; Bon, C.; Delord, K.; Gimenez, O.; Handrich, Y.; Naito, Y.; Guinet, C.; et al. Large-scale climatic anomalies affect marine predator foraging behaviour and demography. *Nat. Commun.* **2015**, *6*, 8220.

(58) Bon, C.; Della Penna, A.; d'Ovidio, F.; Y.P. Arnould, J.; Poupart, T.; Bost, C.-A. Influence of oceanographic structures on foraging strategies: Macaroni penguins at Crozet Islands. *Mov. Ecol.* **2015**, *3* (1), 32.