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Seabird tissues as efficient biomonitoring tools for Hg isotopic investigations: implications of using blood and feathers from chicks and adults

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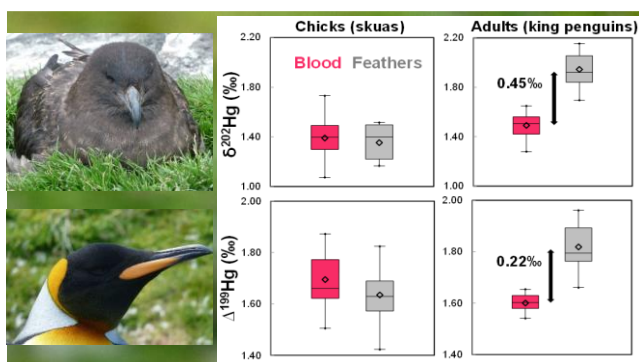
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Abstract

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. $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$) 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 $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ 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 $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ values can be directly compared without any correction factors within and between avian groups. Conversely, penguin adults show higher $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ 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 inter-moult period) Hg accumulation, whereas blood exhibits short-term (i.e. seasonal) Hg exposure in adult birds, the two tissues provide complementary information of 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 pre-industrial 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 blood stream 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 inter-moult 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 synthesise 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, $\delta^{202}\text{Hg}$) and mass-independent fractionation (MIF, $\Delta^{199}\text{Hg}$) 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 ($\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$) 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 correspond 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 hypothesized to induce Hg MDF, leading to differences in $\delta^{202}\text{Hg}$ values between blood and feathers. In contrast, $\Delta^{199}\text{Hg}$ 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 $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ 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.

Material 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) (Figure S1 and Table S1). Ten to eleven birds were randomly chosen in each group. Only one chick per skua nest was sampled to avoid pseudo-replication. 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 was also quantified by using an advanced Hg analyzer (AMA-254, Altec) thus allowing at intercomparing with Hg total concentrations obtained by Hg speciation analyses,

i.e. 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 elsewhere¹⁴. Blood samples analyses were performed as described in a previous study⁴⁰. For Hg speciation analyses, feathers were prepared following a previously developed method¹⁴. Hg was extracted from blood samples (0.10-0.15 g) by alkaline microwave digestion with 5 mL of tetramethylammonium hydroxide (25% TMAH in H₂O, Sigma Aldrich)⁴². Details of the extraction method, analysis and quantification of Hg species are detailed elsewhere¹⁴.

Total Hg isotopic composition analysis

Samples (0.05-0.10 g) were digested with 3 or 5 mL of HNO₃ acid (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-120°C (2°C/min) - 300°C (2.5 h) - 80°C (1 h)). Hotblock mineralization was carried out in Savillex vessels at 75°C during 8 h (6 h in HNO₃ and 2 h more after addition of 1/3 of the total volume of H₂O₂ (30%, ULTREX quality)). Hg isotopic composition was determined using cold-vapor generator (CVG)-MC-ICPMS (Nu Instruments), as detailed previously³⁰. Hg isotopic values were reported as delta notation, calculated relative to the bracketing standard NIST SRM-3133 reference material to allow inter-laboratory comparisons, as described in 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 (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 $98\pm 14\%$ for feathers ($n=104$) and $100\pm 2\%$ 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 validated⁴³. 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 (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 (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 non-parametrical 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 $\alpha=0.05$. 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 ($n = 102$, $92\pm 6\%$ and $92\pm 3\%$, 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 feathers¹⁵. Mean MeHg concentrations differed widely amongst skua chick populations for both blood (0.51 to $3.78 \mu\text{g g}^{-1}$) and feathers (1.82 to $11.78 \mu\text{g g}^{-1}$) and between adult penguin blood (0.43 to $1.95 \mu\text{g g}^{-1}$) and feathers (0.35 to $3.90 \mu\text{g g}^{-1}$) (Figures S2 and S3). Hg species concentrations for each seabird are included in Table S1.

Blood and feather Hg MDF ($\delta^{202}\text{Hg}$) values followed the predicted theoretical MDF line^{47,48} (calculation details in SI). Hg odd-MIF were reported as $\Delta^{199}\text{Hg}$ values, i.e. the difference between measured $\delta^{199}\text{Hg}$ values and $\delta^{199}\text{Hg}$ 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 ($\Delta^{200}\text{Hg}$) - a potential tracer of atmospherically sourced Hg⁴⁹⁻⁵¹ - was also observed (ranging from -0.14 to 0.17 ‰). No significant $\Delta^{200}\text{Hg}$ differences were found between blood and feather samples.

Skua chicks: identical Hg isotopic composition in blood and feathers

Chick blood $\delta^{202}\text{Hg}$ values range from 0.23 ± 0.13 to $1.56\pm 0.08 \text{ ‰}$. Likewise, feather $\delta^{202}\text{Hg}$ values vary widely, from 0.00 ± 0.36 to $1.51\pm 0.20 \text{ ‰}$ (Table 1). Low variability is found in $\Delta^{199}\text{Hg}$ between the four sites, with blood values ranging from 1.51 ± 0.08 to $1.70\pm 0.05 \text{ ‰}$ and feather values varying from 1.46 ± 0.07 to $1.76\pm 0.05 \text{ ‰}$ (Table 1). The range of $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ 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 $\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}$ slope of 1.14 ± 0.07 (Pearson correlation, $R^2=0.86$, $p<0.0001$), while skua chick feathers present a $\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}$ 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

photodemethylation of MeHg (i.e. the magnetic isotope effect)²⁴. No statistical differences of blood and feathers $\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}$ were found amongst the four skua populations. No statistical tissue-specific differences were observed between blood and feather $\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}$ 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 $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ (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 $\delta^{202}\text{Hg}$ values were not overall statistically different in skua chicks except for Kerguelen individuals (Table 1). In the same way, mean $\Delta^{199}\text{Hg}$ paired differences observed for each skua chick population were not statistically significant, except for Amsterdam individuals (Table 1).

Table 1. Blood (red blood cells) and feather $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ values, associated statistics, and isotopic differences between feathers and blood of skua chicks. Both tissues were sampled simultaneously in large chicks. Statistically significant results are marked in bold. Values are means \pm SD.

Species	Location	n	Blood $\delta^{202}\text{Hg}$ (‰)	Feathers $\delta^{202}\text{Hg}$ (‰)	Paired t- test ($\delta^{202}\text{Hg}$ values)		Paired $\delta^{202}\text{Hg}$ differences $\delta^{202}\text{Hg}$ (‰)
			Mean \pm SD	Mean \pm SD	t	p	Mean \pm SD
Antarctic skua	Adélie Land	9	0.23 \pm 0.13	0.00 \pm 0.36	1.455	0.184	-0.16 \pm 0.33
Subantarctic skua	Amsterdam	10	1.56 \pm 0.08	1.51 \pm 0.20	0.884	0.400	-0.06 \pm 0.20
	Crozet	11	1.39 \pm 0.18	1.35 \pm 0.14	-3.209	0.512	-0.04 \pm 0.18
	Kerguelen	10	1.04 \pm 0.08	1.22 \pm 0.19	-0.680	0.009	0.19 \pm 0.18
All populations		40			-0.333	0.741	-0.01 \pm 0.25

Species	Location	n	Blood $\Delta^{199}\text{Hg}$ (‰)	Feathers $\Delta^{199}\text{Hg}$ (‰)	Paired t- test ($\Delta^{199}\text{Hg}$ values)		Paired $\Delta^{199}\text{Hg}$ differences (‰)
			Mean \pm SD	Mean \pm SD	t	p	Mean \pm SD
Antarctic skua	Adélie Land	9	1.51 \pm 0.08	1.46 \pm 0.07	-1.096	0.305	-0.04 \pm 0.11
Subantarctic skua	Amsterdam	10	1.76 \pm 0.05	1.70 \pm 0.05	-2.440	0.037	-0.06 \pm 0.07
	Crozet	11	1.69 \pm 0.11	1.64 \pm 0.12	-1.454	0.180	-0.06 \pm 0.17
	Kerguelen	10	1.61 \pm 0.06	1.55 \pm 0.12	-1.166	0.271	-0.05 \pm 0.12
All populations		40			-2.745	0.008	-0.05 \pm 0.12

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 $\delta^{202}\text{Hg}$ values (Pearson correlation, $R^2=0.91$, $p<0.0001$, $n=40$). $\Delta^{199}\text{Hg}$ values were also significantly correlated but to a lesser extent (Pearson correlation, $R^2=0.53$, $p<0.0001$, $n=40$). Regression slopes were not statistically different from slope 1:1 for $\delta^{202}\text{Hg}$ ($t=-12.68$, $p<0.0001$) and $\Delta^{199}\text{Hg}$ values ($t=-6.54$, $p<0.0001$).

A major result of this study is that skua chicks exhibit identical $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ values in blood and feathers, including similar $\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}$ slopes. This strongly suggests that no metabolic processes influence $\delta^{202}\text{Hg}$ (and $\Delta^{199}\text{Hg}$) during feather growth. Consequently, blood and feathers can be directly compared without applying correction factors, as it is the case for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values⁵³. This finding has important practical consequences for biogeochemical studies using birds as bio-indicators because either feathers or blood can be easily and non-destructively sampled in the field. In other words, the lack of significant differences amongst the two tissues suggests that blood and feather $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ values can be compared confidently within and between various avian data sets that include both chicks and adults.

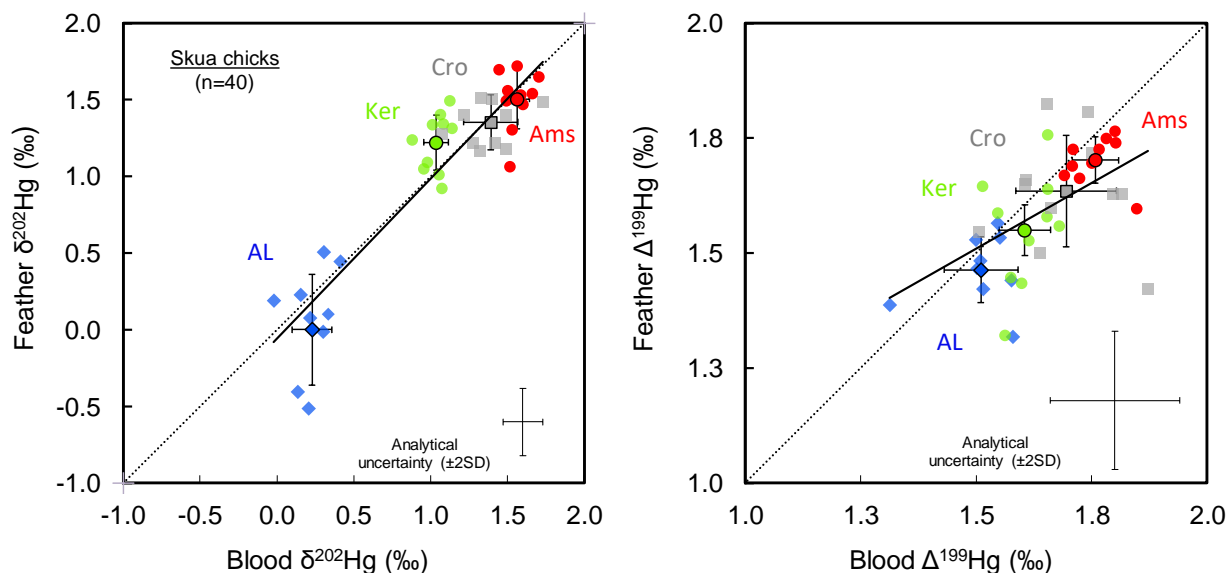


Figure 1. Feather *versus* blood (red blood cells) $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ values in skua chicks (individual values and mean population values). Regression equations for $\delta^{202}\text{Hg}$ blood-feather and $\Delta^{199}\text{Hg}$ blood-feather values are $y=1.12x-0.17$ ($R^2=0.81$, $p<0.0001$), 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).

Penguin adults: Differences in Hg isotopic composition between blood and feathers

Penguin $\delta^{202}\text{Hg}$ values ranged from 0.56 ± 0.20 to 2.16 ± 0.14 ‰ and from 0.67 ± 0.13 to 2.42 ± 0.13 ‰ for blood and feathers, respectively (Table 2). Penguin blood $\Delta^{199}\text{Hg}$ values vary from 1.41 ± 0.06 ‰ to 1.89 ± 0.12 ‰, whereas penguin feathers exhibit slightly higher $\Delta^{199}\text{Hg}$ values that ranged from 1.51 ± 0.12 to 2.22 ± 0.10 ‰ (Table 2). Again, the results obtained for Hg isotopic compositions (both $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ values) are close to those determined in previous studies performed on marine biological organisms^{33,37,38}. Significantly different Hg isotopic values were found amongst the two tissues. Feathers showed greater $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ values than blood in

all species but the gentoo penguin. Respective mean paired differences for $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ were 0.26 ± 0.23 and 0.25 ± 0.13 ‰, respectively (all individuals, $n=62$). However, gentoo penguins show much lower mean paired differences, i.e. -0.02 ± 0.12 ‰ ($\delta^{202}\text{Hg}$) and 0.10 ± 0.12 ‰ ($\Delta^{199}\text{Hg}$), compared to the other penguin species. Paired differences of pooled samples were statistically significant for both $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ values (Table 2). Penguin blood and feather samples displayed $\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}$ 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-MIF²⁴. No statistically different feather $\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}$ ratios were found between penguin species. For blood, no statistical $\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}$ ratios differences were found between penguins, except for NRP ($H=17.38$, $p=0.004$).

There was a high positive correlation among tissues for both $\delta^{202}\text{Hg}$ (Pearson correlation, $R^2=0.93$ $p<0.0001$, $n=62$) and $\Delta^{199}\text{Hg}$ 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 $\delta^{202}\text{Hg}$ ($t=-22.01$, $p<0.0001$) nor for $\Delta^{199}\text{Hg}$ values ($t=-14.41$, $p<0.0001$).

Table 2. Blood (red blood cells) and feather $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ values, associated statistics, and isotopic differences between feathers and blood of adult penguins. Both tissues were sampled simultaneously in adults. Statistically significant results are marked in bold.

Values are means \pm SD

Species	Location	n	Blood $\delta^{202}\text{Hg}$ (‰)	Feathers $\delta^{202}\text{Hg}$ (‰)	Paired t- test ($\delta^{202}\text{Hg}$ values)		Paired $\delta^{202}\text{Hg}$ differences (‰)
			Mean \pm SD	Mean \pm SD	T	P	Mean \pm SD
Adélie penguin	Adélie Land	10	0.56 \pm 0.20	0.67 \pm 0.13	2.147	0.060	0.11 \pm 0.16
Northern rockhopper penguin	Amsterdam	10	2.16 \pm 0.14	2.42 \pm 0.13	5.601	<0.0001	0.27 \pm 0.15
Gentoo penguin	Crozet	11	1.45 \pm 0.12	1.43 \pm 0.10	-0.422	0.682	-0.02 \pm 0.12
King penguin	Crozet	11	1.49 \pm 0.11	1.94 \pm 0.15	7.909	<0.0001	0.45 \pm 0.19
Macaroni penguin	Crozet	10	1.66 \pm 0.11	2.01 \pm 0.15	5.453	<0.0001	0.35 \pm 0.20
Eastern rockhopper penguin	Crozet	10	1.93 \pm 0.18	2.31 \pm 0.12	8.379	<0.0001	0.38 \pm 0.14
All populations		62			-8.795	<0.0001	0.26 \pm 0.23

Species	Location	n	Blood $\Delta^{199}\text{Hg}$ (‰)	Feathers $\Delta^{199}\text{Hg}$ (‰)	Paired t- test ($\Delta^{199}\text{Hg}$ values)		Paired $\Delta^{199}\text{Hg}$ differences (‰)
			Mean \pm SD	Mean \pm SD	T	P	Mean \pm SD
Adélie penguin	Adélie Land	10	1.54 \pm 0.11	1.80 \pm 0.14	8.330	<0.0001	0.26 \pm 0.10
Northern rockhopper penguin	Amsterdam	10	1.89 \pm 0.12	2.22 \pm 0.10	59.061	<0.0001	0.33 \pm 0.08
Gentoo penguin	Crozet	11	1.41 \pm 0.06	1.51 \pm 0.12	2.902	0.016	0.10 \pm 0.12
King penguin	Crozet	11	1.60 \pm 0.04	1.82 \pm 0.09	7.215	<0.0001	0.22 \pm 0.10
Macaroni penguin	Crozet	10	1.54 \pm 0.06	1.86 \pm 0.08	11.410	<0.0001	0.31 \pm 0.09
Eastern rockhopper penguin	Crozet	10	1.77 \pm 0.13	2.09 \pm 0.09	-7.290	<0.0001	0.32 \pm 0.14
All populations		62			-15.390	<0.0001	0.25 \pm 0.13

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 (GP) 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 ($\delta^{202}\text{Hg}$) differences between the two tissues, which is also in agreement with identical blood and feather $\delta^{202}\text{Hg}$ values in skua chicks. Hg MIF is preserved during *in vivo* processes^{26,30}. Hence, $\Delta^{199}\text{Hg}$ 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 $\delta^{202}\text{Hg}$ differences.

Among potential external factors, both temporal and geographical mismatch of Hg integration between blood and feathers could induce Hg isotopic variations for both $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ 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 $\Delta^{199}\text{Hg}$ values than feathers for the same foraging habitat. Unexpectedly, higher $\Delta^{199}\text{Hg}$ 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 spatial³³ and depth scales⁵². Adult seabirds foraging at more opened

oceanic waters, regions distant from the colonies and/or at lower depths of the water column during their inter-breeding period may then present higher $\Delta^{199}\text{Hg}$ values in their feathers than in blood as a consequence of higher light penetration conditions. The very homogeneous values obtained for $\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}$ 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 non-migratory behaviour. Since they stay all year long in coastal waters near the colonies⁵⁵, 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 large areas during the inter-breeding period^{56–58}. Blood isotopic values correspond to the summer breeding period, but feathers also integrate the pre-moulting trip and the inter-breeding migration far way the breeding grounds where birds are likely to feed on different prey with different isotopic $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ values.

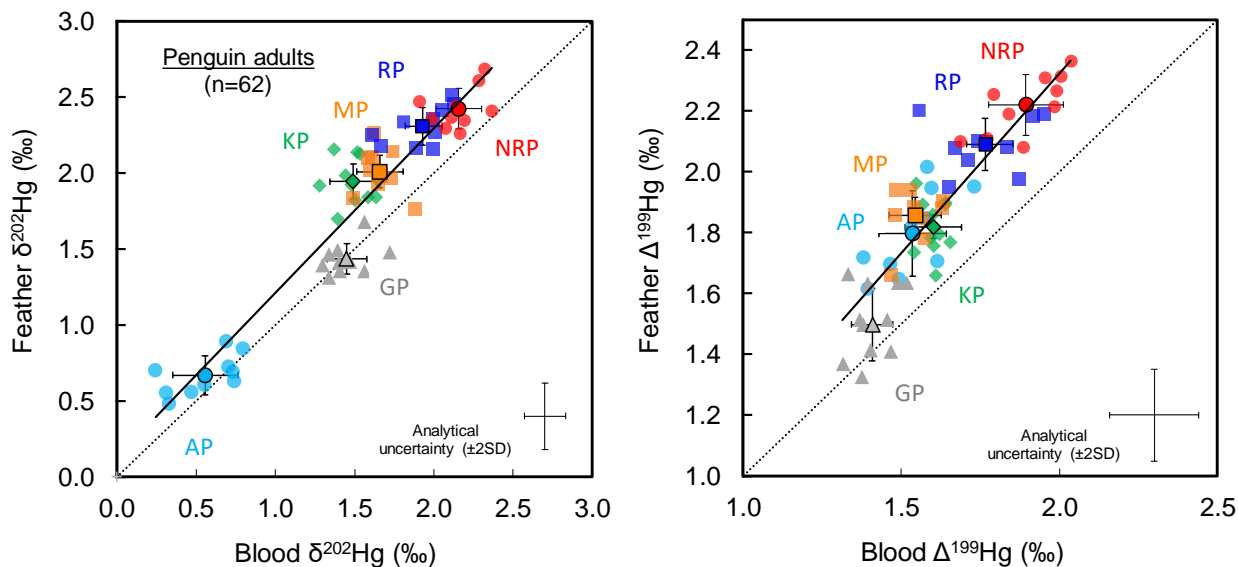


Figure 2. Feather *versus* blood (red blood cells) $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ values in adult penguins (individual values and mean population values). Regression equations for $\delta^{202}\text{Hg}$ blood-feather and $\Delta^{199}\text{Hg}$ blood-feather values are $y=1.08x+0.13$ ($R^2=0.86$, $p<0.0001$) 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).

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 sequester Hg acquired between two moults (approximately 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 $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$) 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 synthesizing 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.

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Supporting information

SI contains supplementary data, figures, tables, methodology and discussion.

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Supporting information

Seabird tissues as efficient biomonitoring tools for Hg isotopic investigations: implications of using blood and feathers from chicks and adults

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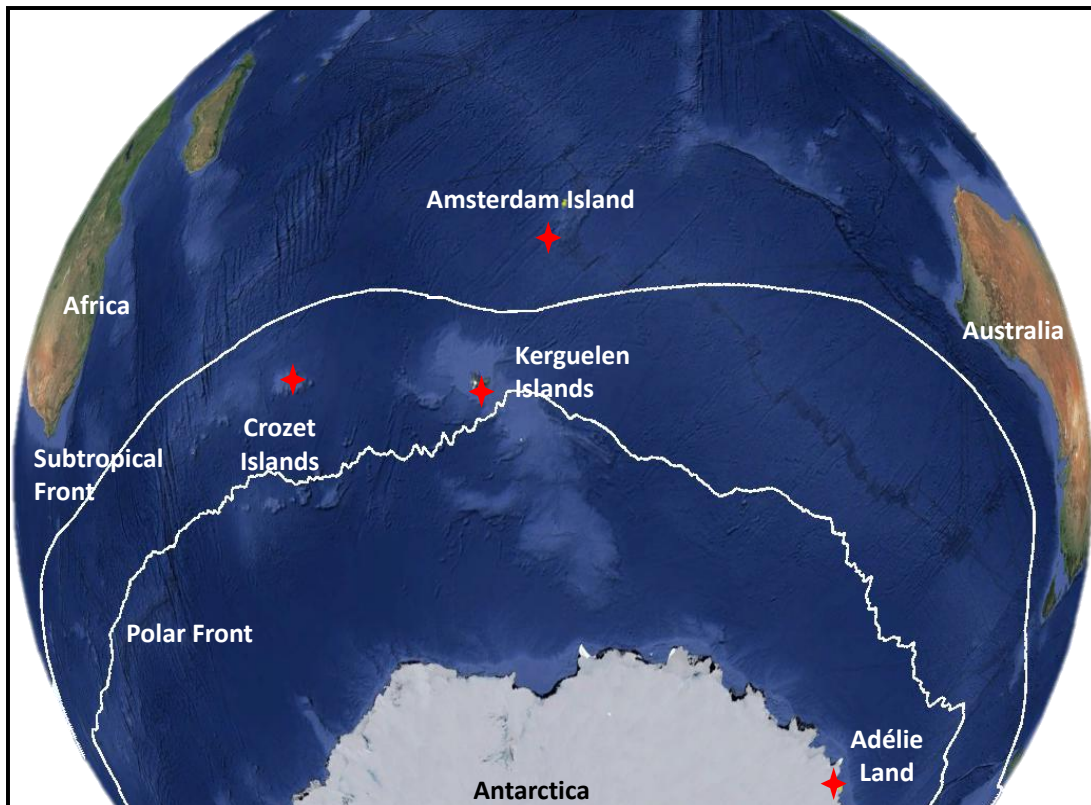


Figure S1. Map of field collection of skua chick and penguin blood and feather samples at the four colony locations of the Southern Ocean: Adélie Land ($66^{\circ}40'S$, $140^{\circ}10'E$), Amsterdam Island ($37^{\circ}50'S$, $77^{\circ}31'E$), Crozet Islands ($46^{\circ}26'S$, $51^{\circ}45'E$) and Kerguelen Islands ($49^{\circ}21'S$, $70^{\circ}18'E$).

Experimental

Hg isotopic composition analyses

Hg isotopic compositions of samples are reported using delta notation according to the following equations:

$$\delta^{xxx} \text{Hg} = \left[\left(\frac{{}^{xxx/198}\text{Hg}_{\text{sample}}}{{}^{xxx/198}\text{Hg}_{\text{NIST 3133}}} \right) - 1 \right] \cdot 1000 (\text{‰}) \quad (1)$$

MIF signatures are expressed using capital delta notation, as suggested elsewhere ¹, according to the following equations:

$$\Delta^{204} \text{Hg} = \delta^{204} \text{Hg} - (1.493 \cdot \delta^{202} \text{Hg}) \quad (2)$$

$$\Delta^{201} \text{Hg} = \delta^{201} \text{Hg} - (0.752 \cdot \delta^{202} \text{Hg}) \quad (3)$$

$$\Delta^{200} \text{Hg} = \delta^{200} \text{Hg} - (0.502 \cdot \delta^{202} \text{Hg}) \quad (4)$$

$$\Delta^{199} \text{Hg} = \delta^{199} \text{Hg} - (0.252 \cdot \delta^{202} \text{Hg}) \quad (5)$$

Table S1. Total Hg, MeHg and iHg concentrations and Hg isotopic composition of blood and feathers of each individual of skua and penguin populations.

Species	Location	Sampling date	Tissue	Individual	MeHg	iHg	THg	$\delta^{204}\text{Hg}$	$\delta^{202}\text{Hg}$	$\delta^{201}\text{Hg}$	$\delta^{200}\text{Hg}$	$\delta^{199}\text{Hg}$	$\Delta^{204}\text{Hg}$	$\Delta^{201}\text{Hg}$	$\Delta^{200}\text{Hg}$	$\Delta^{199}\text{Hg}$
					($\mu\text{g g}^{-1}$)	($\mu\text{g g}^{-1}$)	($\mu\text{g g}^{-1}$)	‰	‰	‰	‰	‰	‰	‰	‰	
Antarctic skua	Adélie Land	Dec 2011-Jan 2012	Feathers	ASP 01	2.01	0.34	2.34	0.75	0.51	1.76	0.24	1.61	-0.01	1.37	-0.01	1.48
Antarctic skua	Adélie Land	Dec 2011-Jan 2012	Feathers	ASP 02	1.62	0.30	1.92	0.29	0.19	1.54	0.15	1.58	0.00	1.40	0.05	1.53
Antarctic skua	Adélie Land	Dec 2011-Jan 2012	Feathers	ASP 03	1.73	0.13	1.86	0.22	0.08	1.39	0.07	1.55	0.10	1.33	0.03	1.53
Antarctic skua	Adélie Land	Dec 2011-Jan 2012	Feathers	ASP 04	2.37	0.15	2.39	-0.21	-0.40	0.94	-0.21	1.34	0.40	1.24	-0.01	1.44
Antarctic skua	Adélie Land	Dec 2011-Jan 2012	Feathers	ASP 05	1.29	0.12	1.34	0.27	0.10	1.22	-0.04	1.49	0.12	1.14	-0.09	1.47
Antarctic skua	Adélie Land	Dec 2011-Jan 2012	Feathers	ASP 06	1.80	0.12	1.84	-0.55	-0.56	0.89	-0.23	1.25	0.28	1.31	0.05	1.39
Antarctic skua	Adélie Land	Dec 2011-Jan 2012	Feathers	ASP 07	1.84	0.13	1.89	-0.07	-0.05	1.23	-0.11	1.49	0.00	1.26	-0.08	1.50
Antarctic skua	Adélie Land	Dec 2011-Jan 2012	Feathers	ASP 08	1.85	0.11	1.96	0.32	0.23	1.42	0.10	1.51	-0.02	1.24	-0.01	1.46
Antarctic skua	Adélie Land	Dec 2011-Jan 2012	Feathers	ASP 09	1.70	0.13	1.75	-0.57	-0.51	0.96	-0.27	1.19	0.19	1.34	-0.01	1.32
Antarctic skua	Adélie Land	Dec 2011-Jan 2012	Feathers	ASP 10	1.98	0.19	2.08	0.07	-0.01	1.28	0.03	1.42	0.09	1.29	0.04	1.42
Antarctic skua	Adélie Land	Dec 2011-Jan 2012	Feathers	ASP 11	1.80	0.10	1.89	0.64	0.45	1.70	0.23	1.68	-0.03	1.37	0.00	1.57
Antarctic skua	Adélie Land	Dec 2011-Jan 2012	Blood	ASP 01	0.56	0.03	0.59	0.55	0.31	1.53	0.13	1.59	0.09	1.30	-0.02	1.51
Antarctic skua	Adélie Land	Dec 2011-Jan 2012	Blood	ASP 02	0.55	0.06	0.61	0.08	-0.02	1.32	0.00	1.55	0.11	1.33	0.01	1.55
Antarctic skua	Adélie Land	Dec 2011-Jan 2012	Blood	ASP 03	0.56	0.03	0.59	0.19	0.22	1.41	0.07	1.55	-0.13	1.25	-0.04	1.50
Antarctic skua	Adélie Land	Dec 2011-Jan 2012	Blood	ASP 04	0.39	0.05	0.44	0.23	0.13	1.49	0.03	1.61	0.03	1.38	-0.04	1.58
Antarctic skua	Adélie Land	Dec 2011-Jan 2012	Blood	ASP 05	0.38	0.05	0.43	0.50	0.34	1.51	0.19	1.59	0.00	1.25	0.02	1.50
Antarctic skua	Adélie Land	Dec 2011-Jan 2012	Blood	ASP 06	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Antarctic skua	Adélie Land	Dec 2011-Jan 2012	Blood	ASP 07	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Antarctic skua	Adélie Land	Dec 2011-Jan 2012	Blood	ASP 08	0.40	0.09	0.50	0.29	0.16	1.23	0.03	1.35	0.05	1.12	-0.05	1.31
Antarctic skua	Adélie Land	Dec 2011-Jan 2012	Blood	ASP 09	0.40	0.05	0.45	0.36	0.21	1.48	0.13	1.63	0.05	1.33	0.02	1.58

Antarctic skua	Adélie Land	Dec 2011-Jan 2012	Blood	ASP 10	0.49	0.06	0.55	0.45	0.30	1.48	0.12	1.59	0.01	1.26	-0.03	1.52
Antarctic skua	Adélie Land	Dec 2011-Jan 2012	Blood	ASP 11	0.59	0.06	0.65	0.53	0.41	1.60	0.22	1.65	-0.09	1.28	0.01	1.55
Subantarctic skua	Amsterdam	Dec 2011	Feathers	SSAMS12P01	13.38	0.75	14.13	2.36	1.56	2.61	0.83	2.13	0.02	1.44	0.05	1.74
Subantarctic skua	Amsterdam	Dec 2011	Feathers	SSAMS12P02	16.95	0.71	17.67	2.47	1.72	2.71	0.93	2.18	-0.11	1.42	0.06	1.75
Subantarctic skua	Amsterdam	Dec 2011	Feathers	SSAMS12P03	10.67	0.63	11.31	2.43	1.70	2.70	0.92	2.12	-0.10	1.43	0.07	1.70
Subantarctic skua	Amsterdam	Dec 2011	Feathers	SSAMS12P04	11.47	0.43	11.90	2.20	1.47	2.40	0.78	1.97	0.00	1.30	0.04	1.60
Subantarctic skua	Amsterdam	Dec 2011	Feathers	SSAMS12P05	12.34	0.59	12.93	1.48	1.07	2.17	0.51	1.94	-0.11	1.36	-0.03	1.67
Subantarctic skua	Amsterdam	Dec 2011	Feathers	SSAMS12P06	13.61	0.51	14.12	2.35	1.65	2.67	0.92	2.11	-0.12	1.42	0.09	1.69
Subantarctic skua	Amsterdam	Dec 2011	Feathers	SSAMS12P07	8.90	0.37	9.27	2.32	1.54	2.68	0.87	2.12	0.02	1.52	0.10	1.73
Subantarctic skua	Amsterdam	Dec 2011	Feathers	SSAMS12P08	10.44	0.68	11.12	2.14	1.49	2.52	0.76	2.10	-0.09	1.40	0.01	1.73
Subantarctic skua	Amsterdam	Dec 2011	Feathers	SSAMS12P09	9.67	0.47	10.15	2.26	1.53	2.59	0.78	2.15	-0.03	1.43	0.01	1.76
Subantarctic skua	Amsterdam	Dec 2011	Feathers	SSAMS12P10	10.38	0.69	11.07	1.89	1.31	2.34	0.69	1.99	-0.07	1.35	0.03	1.66
Subantarctic skua	Amsterdam	Dec 2011	Blood	SSAMS12P01	3.30	0.04	3.34	2.21	1.50	2.60	0.80	2.18	-0.03	1.47	0.05	1.80
Subantarctic skua	Amsterdam	Dec 2011	Blood	SSAMS12P02	4.27	0.06	4.33	2.32	1.56	2.67	0.83	2.18	-0.01	1.49	0.05	1.78
Subantarctic skua	Amsterdam	Dec 2011	Blood	SSAMS12P03	3.96	0.55	4.51	2.26	1.44	2.55	0.82	2.11	0.10	1.46	0.09	1.75
Subantarctic skua	Amsterdam	Dec 2011	Blood	SSAMS12P04	3.28	0.16	3.44	2.33	1.60	2.68	0.91	2.25	-0.05	1.48	0.11	1.85
Subantarctic skua	Amsterdam	Dec 2011	Blood	SSAMS12P05	4.47	0.47	4.94	2.18	1.52	2.54	0.71	2.07	-0.08	1.40	-0.05	1.69
Subantarctic skua	Amsterdam	Dec 2011	Blood	SSAMS12P06	5.54	0.28	5.82	2.40	1.71	2.72	0.85	2.14	-0.15	1.44	-0.01	1.71
Subantarctic skua	Amsterdam	Dec 2011	Blood	SSAMS12P07	2.93	0.07	3.00	2.39	1.66	2.74	0.85	2.18	-0.09	1.49	0.01	1.77
Subantarctic skua	Amsterdam	Dec 2011	Blood	SSAMS12P08	3.47	0.34	3.81	2.21	1.49	2.56	0.75	2.09	-0.02	1.44	0.00	1.71
Subantarctic skua	Amsterdam	Dec 2011	Blood	SSAMS12P09	3.31	0.12	3.43	2.48	1.59	2.75	0.82	2.20	0.11	1.56	0.02	1.80
Subantarctic skua	Amsterdam	Dec 2011	Blood	SSAMS12P10	3.24	0.14	3.38	2.30	1.53	2.63	0.80	2.11	0.01	1.48	0.03	1.72
Subantarctic skua	Crozet	Jan-Feb 2012	Feathers	SSCROP01	5.76	0.36	6.12	2.39	1.51	2.57	0.81	2.03	0.14	1.44	0.06	1.65
Subantarctic skua	Crozet	Jan-Feb 2012	Feathers	SSCROP02	5.78	0.33	6.10	1.73	1.17	2.15	0.59	1.84	-0.01	1.27	0.00	1.55
Subantarctic skua	Crozet	Jan-Feb 2012	Feathers	SSCROP03	6.10	0.27	6.33	2.40	1.52	2.52	0.81	1.98	0.14	1.38	0.05	1.60

Subantarctic skua	Crozet	Jan-Feb 2012	Feathers	SSCROP04	5.61	0.71	6.32	1.77	1.18	2.17	0.54	1.80	0.01	1.28	-0.05	1.50
Subantarctic skua	Crozet	Jan-Feb 2012	Feathers	SSCROP05	5.73	0.19	5.91	2.09	1.40	2.46	0.60	2.01	0.00	1.41	-0.10	1.66
Subantarctic skua	Crozet	Jan-Feb 2012	Feathers	SSCROP06	2.97	0.14	3.11	2.39	1.51	2.43	0.78	2.01	0.14	1.29	0.02	1.63
Subantarctic skua	Crozet	Jan-Feb 2012	Feathers	SSCROP07	5.13	0.26	5.38	2.42	1.48	2.53	0.74	2.00	0.20	1.42	-0.01	1.63
Subantarctic skua	Crozet	Jan-Feb 2012	Feathers	SSCROP08	2.84	0.14	2.98	1.90	1.28	2.49	0.62	2.13	-0.01	1.53	-0.02	1.81
Subantarctic skua	Crozet	Jan-Feb 2012	Feathers	SSCROP09	5.81	0.37	6.18	1.91	1.22	2.15	0.65	1.73	0.09	1.23	0.03	1.42
Subantarctic skua	Crozet	Jan-Feb 2012	Feathers	SSCROP10	2.18	0.11	2.29	1.84	1.22	2.47	0.50	2.13	0.02	1.56	-0.11	1.82
Subantarctic skua	Crozet	Jan-Feb 2012	Feathers	SSCROP11	3.15	0.13	3.29	2.04	1.40	2.51	0.76	2.07	-0.06	1.45	0.05	1.72
Subantarctic skua	Crozet	Jan-Feb 2012	Blood	SSCROP01	2.10	0.33	2.43	2.14	1.40	2.45	0.71	1.96	0.05	1.40	0.01	1.60
Subantarctic skua	Crozet	Jan-Feb 2012	Blood	SSCROP02	2.91	0.05	2.96	2.02	1.32	2.34	0.68	1.84	0.04	1.35	0.02	1.51
Subantarctic skua	Crozet	Jan-Feb 2012	Blood	SSCROP03	1.07	0.07	1.14	1.86	1.33	2.42	0.73	2.00	-0.12	1.42	0.06	1.66
Subantarctic skua	Crozet	Jan-Feb 2012	Blood	SSCROP04	4.38	0.13	4.52	2.20	1.49	2.46	0.76	2.01	-0.03	1.34	0.02	1.64
Subantarctic skua	Crozet	Jan-Feb 2012	Blood	SSCROP05	2.50	0.19	2.68	1.93	1.22	2.28	0.59	1.91	0.11	1.36	-0.02	1.61
Subantarctic skua	Crozet	Jan-Feb 2012	Blood	SSCROP06	0.67	0.07	0.74	2.19	1.56	2.65	0.77	2.21	-0.13	1.48	-0.01	1.82
Subantarctic skua	Crozet	Jan-Feb 2012	Blood	SSCROP07	1.09	0.08	1.18	2.54	1.73	2.83	0.92	2.23	-0.04	1.52	0.05	1.79
Subantarctic skua	Crozet	Jan-Feb 2012	Blood	SSCROP08	0.88	0.05	0.93	1.64	1.07	2.34	0.55	2.01	0.04	1.54	0.02	1.74
Subantarctic skua	Crozet	Jan-Feb 2012	Blood	SSCROP09	1.17	0.08	1.25	2.02	1.42	2.62	0.77	2.23	-0.10	1.55	0.06	1.87
Subantarctic skua	Crozet	Jan-Feb 2012	Blood	SSCROP10	0.89	0.04	0.93	1.92	1.27	2.29	0.69	1.97	0.02	1.34	0.05	1.65
Subantarctic skua	Crozet	Jan-Feb 2012	Blood	SSCROP11	1.02	0.07	1.09	2.26	1.49	2.66	0.75	2.13	0.03	1.54	0.00	1.75
Subantarctic skua	Kerguelen	Dec 2011	Feathers	SSKERP01	6.20	0.28	6.48	1.67	1.34	2.53	0.78	2.10	-0.34	1.52	0.11	1.76
Subantarctic skua	Kerguelen	Dec 2011	Feathers	SSKERP02	3.92	0.27	4.13	1.29	1.02	2.16	0.60	1.84	-0.22	1.39	0.09	1.58
Subantarctic skua	Kerguelen	Dec 2011	Feathers	SSKERP03	7.16	0.52	7.68	1.66	1.05	2.13	0.52	1.82	0.09	1.34	-0.01	1.56
Subantarctic skua	Kerguelen	Dec 2011	Feathers	SSKERP04	5.93	0.48	6.41	1.96	1.24	2.29	0.62	1.75	0.11	1.36	0.00	1.43
Subantarctic skua	Kerguelen	Dec 2011	Feathers	SSKERP05	7.12	0.62	7.70	2.12	1.50	2.51	0.83	2.02	-0.11	1.39	0.07	1.65
Subantarctic skua	Kerguelen	Dec 2011	Feathers	SSKERP06	7.43	0.47	7.90	1.86	1.34	2.31	0.71	1.93	-0.14	1.30	0.04	1.59

Subantarctic skua	Kerguelen	Dec 2011	Feathers	SSKERP07	6.88	0.31	7.19	1.49	0.93	2.04	0.49	1.68	0.11	1.34	0.02	1.45
Subantarctic skua	Kerguelen	Dec 2011	Feathers	SSKERP08	6.39	0.77	7.09	1.85	1.09	2.13	0.51	1.60	0.21	1.31	-0.04	1.32
Subantarctic skua	Kerguelen	Dec 2011	Feathers	SSKERP09	7.74	0.47	8.21	1.81	1.32	2.30	0.71	1.86	-0.15	1.31	0.05	1.53
Subantarctic skua	Kerguelen	Dec 2011	Feathers	SSKERP10	6.19	0.81	6.99	2.03	1.40	2.38	0.71	1.99	-0.06	1.32	0.00	1.64
Subantarctic skua	Kerguelen	Dec 2011	Blood	SSKERP01	2.37	0.11	2.48	1.54	1.08	2.20	0.54	1.93	-0.08	1.38	-0.01	1.65
Subantarctic skua	Kerguelen	Dec 2011	Blood	SSKERP02	1.83	0.11	1.94	1.64	1.05	2.19	0.50	1.92	0.06	1.39	-0.03	1.65
Subantarctic skua	Kerguelen	Dec 2011	Blood	SSKERP03	2.03	0.11	2.14	1.46	0.96	2.16	0.49	1.92	0.03	1.44	0.01	1.68
Subantarctic skua	Kerguelen	Dec 2011	Blood	SSKERP04	1.78	0.25	2.03	1.36	0.88	2.06	0.41	1.82	0.05	1.40	-0.03	1.60
Subantarctic skua	Kerguelen	Dec 2011	Blood	SSKERP05	2.50	0.18	2.69	1.60	1.12	2.20	0.53	1.80	-0.07	1.36	-0.04	1.51
Subantarctic skua	Kerguelen	Dec 2011	Blood	SSKERP06	2.22	0.14	2.37	1.60	1.01	2.07	0.55	1.80	0.09	1.31	0.04	1.55
Subantarctic skua	Kerguelen	Dec 2011	Blood	SSKERP07	1.64	0.13	1.77	1.55	1.07	2.13	0.57	1.85	-0.05	1.33	0.03	1.58
Subantarctic skua	Kerguelen	Dec 2011	Blood	SSKERP08	2.30	0.17	2.47	1.45	0.98	2.05	0.46	1.81	-0.02	1.32	-0.03	1.56
Subantarctic skua	Kerguelen	Dec 2011	Blood	SSKERP09	2.62	0.21	2.83	1.74	1.14	2.25	0.53	1.90	0.04	1.40	-0.04	1.61
Subantarctic skua	Kerguelen	Dec 2011	Blood	SSKERP10	2.32	0.19	2.51	1.53	1.06	2.24	0.59	1.92	-0.06	1.44	0.06	1.65
Adélie penguin	Adélie Land	Feb 2012	Feathers	APA11	0.33	0.01	0.33	1.37	0.89	2.36	0.39	2.17	0.04	1.69	-0.06	1.95
Adélie penguin	Adélie Land	Feb 2012	Feathers	APA12	0.28	0.01	0.29	0.81	0.48	2.11	0.18	2.14	0.10	1.75	-0.06	2.02
Adélie penguin	Adélie Land	Feb 2012	Feathers	APA13	0.29	0.03	0.33	0.97	0.63	1.94	0.24	1.87	0.03	1.46	-0.07	1.71
Adélie penguin	Adélie Land	Feb 2012	Feathers	APA14	0.32	0.03	0.35	0.83	0.56	1.90	0.24	1.86	0.00	1.48	-0.04	1.72
Adélie penguin	Adélie Land	Feb 2012	Feathers	APA15	0.29	0.02	0.30	1.11	0.69	1.98	0.38	1.87	0.07	1.46	0.03	1.70
Adélie penguin	Adélie Land	Feb 2012	Feathers	APA16	0.38	0.04	0.42	1.26	0.85	2.12	0.40	2.06	-0.01	1.49	-0.02	1.85
Adélie penguin	Adélie Land	Feb 2012	Feathers	APA17	0.28	0.01	0.29	0.84	0.60	2.12	0.36	2.11	-0.06	1.67	0.06	1.95
Adélie penguin	Adélie Land	Feb 2012	Feathers	APA18	0.34	0.05	0.39	0.90	0.56	2.02	0.18	1.96	0.07	1.60	-0.10	1.82
Adélie penguin	Adélie Land	Feb 2012	Feathers	APA19	0.47	0.04	0.51	0.98	0.72	1.90	0.40	1.83	-0.10	1.36	0.03	1.65
Adélie penguin	Adélie Land	Feb 2012	Feathers	APA20	0.49	0.05	0.53	1.04	0.70	1.90	0.41	1.79	-0.01	1.37	0.05	1.62
Adélie penguin	Adélie Land	Feb 2012	Blood	APA11	0.42	0.03	0.45	1.03	0.69	1.87	0.33	1.77	0.00	1.35	-0.02	1.60

Adélie penguin	Adélie Land	Feb 2012	Blood	APA12	0.38	0.05	0.42	0.52	0.33	1.78	0.13	1.66	0.03	1.53	-0.04	1.58
Adélie penguin	Adélie Land	Feb 2012	Blood	APA13	0.48	0.01	0.50	1.14	0.74	1.92	0.38	1.80	0.03	1.36	0.01	1.61
Adélie penguin	Adélie Land	Feb 2012	Blood	APA14	0.38	0.02	0.40	0.45	0.31	1.41	0.13	1.46	-0.01	1.17	-0.03	1.38
Adélie penguin	Adélie Land	Feb 2012	Blood	APA15	0.48	0.16	0.64	1.02	0.73	1.77	0.39	1.65	-0.07	1.22	0.03	1.47
Adélie penguin	Adélie Land	Feb 2012	Blood	APA16	0.46	0.03	0.49	1.09	0.79	1.85	0.35	1.78	-0.10	1.25	-0.05	1.58
Adélie penguin	Adélie Land	Feb 2012	Blood	APA17	0.18	0.00	0.18	0.71	0.56	2.00	0.14	1.87	-0.12	1.58	-0.14	1.73
Adélie penguin	Adélie Land	Feb 2012	Blood	APA18	0.56	0.02	0.58	0.77	0.47	1.66	0.26	1.65	0.07	1.30	0.02	1.53
Adélie penguin	Adélie Land	Feb 2012	Blood	APA19	0.52	0.02	0.55	1.10	0.71	1.86	0.30	1.67	0.04	1.33	-0.06	1.49
Adélie penguin	Adélie Land	Feb 2012	Blood	APA20	0.45	0.01	0.45	0.37	0.24	1.41	0.09	1.45	0.01	1.23	-0.04	1.39
Northern rockhopper penguin	Amsterdam	Nov 2011	Feathers	NRPA11	1.55	0.20	1.75	3.47	2.35	3.57	1.17	2.70	-0.03	1.81	0.00	2.11
Northern rockhopper penguin	Amsterdam	Nov 2011	Feathers	NRPA12	1.40	0.19	1.59	3.54	2.36	3.81	1.12	2.96	0.02	2.04	-0.06	2.36
Northern rockhopper penguin	Amsterdam	Nov 2011	Feathers	NRPA13	1.16	0.09	1.25	3.63	2.47	3.82	1.28	2.93	-0.05	1.96	0.04	2.31
Northern rockhopper penguin	Amsterdam	Nov 2011	Feathers	NRPA14	1.36	0.15	1.51	3.77	2.44	3.80	1.23	2.87	0.12	1.97	0.01	2.25
Northern rockhopper penguin	Amsterdam	Nov 2011	Feathers	NRPA15	1.55	0.08	1.63	3.53	2.36	3.70	1.22	2.91	0.00	1.92	0.03	2.31
Northern rockhopper penguin	Amsterdam	Nov 2011	Feathers	NRPA16	1.93	0.10	2.03	3.82	2.61	3.77	1.32	2.74	-0.07	1.81	0.01	2.08
Northern rockhopper penguin	Amsterdam	Nov 2011	Feathers	NRPA17	1.61	0.11	1.72	3.41	2.29	3.66	1.30	2.84	-0.01	1.94	0.15	2.27
Northern rockhopper penguin	Amsterdam	Nov 2011	Feathers	NRPA18	1.80	0.09	1.89	4.01	2.68	3.89	1.40	2.89	0.01	1.87	0.05	2.21
Northern rockhopper penguin	Amsterdam	Nov 2011	Feathers	NRPA19	1.47	0.09	1.56	3.58	2.41	3.65	1.20	2.80	-0.02	1.84	-0.01	2.19
Northern rockhopper penguin	Amsterdam	Nov 2011	Feathers	NRPA20	1.88	0.13	2.01	3.39	2.26	3.55	1.11	2.67	0.02	1.85	-0.03	2.10
Northern rockhopper penguin	Amsterdam	Nov 2011	Blood	NRPA11	2.11	0.80	2.90	3.28	2.19	3.15	1.10	2.32	0.01	1.50	0.00	1.77
Northern rockhopper penguin	Amsterdam	Nov 2011	Blood	NRPA12	1.38	0.82	2.20	2.91	1.99	3.19	1.00	2.54	-0.05	1.70	0.00	2.04
Northern rockhopper penguin	Amsterdam	Nov 2011	Blood	NRPA13	1.68	0.15	1.83	2.87	1.91	3.02	1.02	2.49	0.02	1.59	0.06	2.00
Northern rockhopper penguin	Amsterdam	Nov 2011	Blood	NRPA14	2.42	1.08	3.50	3.19	2.14	3.06	1.14	2.33	-0.01	1.45	0.06	1.79
Northern rockhopper penguin	Amsterdam	Nov 2011	Blood	NRPA15	1.46	0.16	1.62	3.01	2.11	3.22	1.08	2.49	-0.14	1.64	0.02	1.95
Northern rockhopper penguin	Amsterdam	Nov 2011	Blood	NRPA16	2.19	0.13	2.32	3.44	2.28	3.27	1.23	2.46	0.03	1.55	0.08	1.89

Northern rockhopper penguin	Amsterdam	Nov 2011	Blood	NRPA17	1.48	0.72	2.20	3.15	2.08	3.12	1.16	2.51	0.05	1.56	0.12	1.99
Northern rockhopper penguin	Amsterdam	Nov 2011	Blood	NRPA18	2.51	0.26	2.77	3.63	2.32	3.28	1.33	2.57	0.16	1.53	0.17	1.98
Northern rockhopper penguin	Amsterdam	Nov 2011	Blood	NRPA19	1.84	0.17	2.01	3.45	2.37	3.38	1.14	2.44	-0.09	1.60	-0.05	1.84
Northern rockhopper penguin	Amsterdam	Nov 2011	Blood	NRPA20	2.41	0.18	2.59	3.19	2.17	3.02	1.11	2.23	-0.05	1.39	0.02	1.69
King penguin	Crozet	Oct 2011	Feathers	KPA01	2.29	0.19	2.48	3.08	1.95	3.15	1.00	2.35	0.16	1.68	0.02	1.86
King penguin	Crozet	Oct 2011	Feathers	KPA02	1.84	0.14	1.97	2.83	1.82	2.92	0.91	2.19	0.11	1.55	0.00	1.73
King penguin	Crozet	Oct 2011	Feathers	KPA03	1.54	0.12	1.66	2.95	1.92	3.16	0.97	2.38	0.08	1.72	0.00	1.90
King penguin	Crozet	Oct 2011	Feathers	KPA04	1.84	0.15	1.98	3.19	2.14	3.27	1.08	2.43	0.00	1.67	0.01	1.89
King penguin	Crozet	Oct 2011	Feathers	KPA05	1.97	0.16	2.13	2.88	1.92	3.12	0.99	2.44	0.02	1.68	0.02	1.96
King penguin	Crozet	Oct 2011	Feathers	KPA06	2.77	0.18	2.95	2.72	1.84	3.03	0.95	2.36	-0.03	1.65	0.02	1.89
King penguin	Crozet	Oct 2011	Feathers	KPA07	1.81	0.22	2.03	3.20	2.13	3.18	1.05	2.32	0.02	1.58	-0.01	1.78
King penguin	Crozet	Oct 2011	Feathers	KPA08	1.89	0.20	2.08	3.00	1.98	3.02	1.01	2.26	0.04	1.53	0.01	1.76
King penguin	Crozet	Oct 2011	Feathers	KPA09	3.74	0.40	4.14	2.57	1.70	2.74	0.86	2.09	0.03	1.46	0.01	1.66
King penguin	Crozet	Oct 2011	Feathers	KPA10	1.80	0.13	1.93	3.18	2.15	3.20	1.05	2.34	-0.04	1.58	-0.04	1.79
King penguin	Crozet	Oct 2011	Feathers	KPA11	1.76	0.08	1.84	2.66	1.84	2.84	0.93	2.23	-0.09	1.45	0.01	1.77
King penguin	Crozet	Oct 2011	Blood	KPA01	1.71	0.14	1.85	2.51	1.65	2.60	0.81	2.02	0.05	1.36	-0.02	1.60
King penguin	Crozet	Oct 2011	Blood	KPA02	2.04	0.14	2.19	2.12	1.51	2.47	0.70	1.92	-0.13	1.34	-0.05	1.54
King penguin	Crozet	Oct 2011	Blood	KPA03	1.63	0.11	1.73	2.24	1.48	2.49	0.71	2.01	0.02	1.38	-0.03	1.64
King penguin	Crozet	Oct 2011	Blood	KPA04	1.73	0.13	1.87	2.29	1.52	2.52	0.79	2.02	0.02	1.37	0.03	1.64
King penguin	Crozet	Oct 2011	Blood	KPA05	1.96	0.16	2.12	1.85	1.28	2.37	0.61	1.87	-0.06	1.40	-0.03	1.55
King penguin	Crozet	Oct 2011	Blood	KPA06	1.80	0.10	1.90	2.17	1.59	2.60	0.76	1.97	-0.20	1.41	-0.03	1.57
King penguin	Crozet	Oct 2011	Blood	KPA07	2.44	0.16	2.60	2.14	1.53	2.50	0.78	1.98	-0.16	1.35	0.01	1.59
King penguin	Crozet	Oct 2011	Blood	KPA08	1.57	0.12	1.70	2.12	1.45	2.50	0.71	1.97	-0.04	1.41	-0.02	1.60
King penguin	Crozet	Oct 2011	Blood	KPA09	1.91	0.12	2.02	2.12	1.40	2.38	0.71	1.96	0.03	1.33	0.00	1.61
King penguin	Crozet	Oct 2011	Blood	KPA10	2.26	0.15	2.42	2.10	1.37	2.45	0.69	1.97	0.05	1.41	0.00	1.62

King penguin	Crozet	Oct 2011	Blood	KPA11	1.65	0.11	1.77	2.33	1.64	2.61	0.77	2.07	-0.12	1.38	-0.06	1.65
Gentoo penguin	Crozet	Oct 2011	Feathers	GPA01	6.84	0.20	7.59	2.14	1.48	2.28	0.71	1.78	-0.07	1.17	-0.03	1.41
Gentoo penguin	Crozet	Oct 2011	Feathers	GPA02	3.90	0.20	4.30	2.20	1.41	2.26	0.68	1.73	0.09	1.19	-0.03	1.37
Gentoo penguin	Crozet	Oct 2011	Feathers	GPA03	3.21	0.22	3.62	2.59	1.68	2.74	0.80	2.06	0.08	1.48	-0.05	1.64
Gentoo penguin	Crozet	Oct 2011	Feathers	GPA04	6.14	0.25	6.77	2.05	1.35	2.24	0.61	1.67	0.03	1.22	-0.07	1.33
Gentoo penguin	Crozet	Oct 2011	Feathers	GPA05	3.75	0.16	4.08	2.09	1.44	2.34	0.71	1.86	-0.05	1.26	-0.02	1.50
Gentoo penguin	Crozet	Oct 2011	Feathers	GPA06	1.39	0.26	1.55	2.03	1.31	2.39	0.72	1.97	0.07	1.40	0.06	1.63
Gentoo penguin	Crozet	Oct 2011	Feathers	GPA07	4.39	0.23	4.81	2.04	1.36	2.19	0.72	1.76	0.02	1.17	0.04	1.42
Gentoo penguin	Crozet	Oct 2011	Feathers	GPA08	4.17	0.20	4.81	2.14	1.49	2.34	0.73	1.89	-0.09	1.21	-0.02	1.51
Gentoo penguin	Crozet	Oct 2011	Feathers	GPA09	1.98	0.33	2.25	2.22	1.46	2.53	0.71	2.03	0.04	1.43	-0.03	1.66
Gentoo penguin	Crozet	Oct 2011	Feathers	GPA10	4.93	0.17	5.41	2.11	1.41	2.33	0.70	1.87	0.01	1.27	-0.01	1.51
Gentoo penguin	Crozet	Oct 2011	Feathers	GPA11	2.19	0.75	2.46	2.17	1.39	2.46	0.71	1.98	0.09	1.42	0.01	1.63
Gentoo penguin	Crozet	Oct 2011	Blood	GPA01	3.50	0.29	3.79	2.57	1.72	2.52	0.83	1.90	0.00	1.23	-0.03	1.47
Gentoo penguin	Crozet	Oct 2011	Blood	GPA02	1.94	0.15	2.09	2.22	1.47	2.28	0.74	1.68	0.02	1.17	0.00	1.31
Gentoo penguin	Crozet	Oct 2011	Blood	GPA03	1.32	0.09	1.40	2.35	1.56	2.46	0.76	1.91	0.01	1.28	-0.03	1.52
Gentoo penguin	Crozet	Oct 2011	Blood	GPA04	3.47	0.24	3.71	2.16	1.41	2.28	0.74	1.73	0.06	1.22	0.03	1.37
Gentoo penguin	Crozet	Oct 2011	Blood	GPA05	1.23	0.10	1.33	2.24	1.45	2.29	0.74	1.75	0.08	1.20	0.01	1.38
Gentoo penguin	Crozet	Oct 2011	Blood	GPA06	1.13	0.11	1.25	1.96	1.34	2.23	0.65	1.83	-0.04	1.23	-0.03	1.49
Gentoo penguin	Crozet	Oct 2011	Blood	GPA07	1.47	0.11	1.58	2.37	1.57	2.35	0.79	1.80	0.04	1.17	0.00	1.40
Gentoo penguin	Crozet	Oct 2011	Blood	GPA08	1.54	0.11	1.64	2.13	1.39	2.25	0.65	1.72	0.05	1.20	-0.05	1.37
Gentoo penguin	Crozet	Oct 2011	Blood	GPA09	1.10	0.10	1.19	2.03	1.34	2.14	0.67	1.67	0.03	1.13	-0.01	1.33
Gentoo penguin	Crozet	Oct 2011	Blood	GPA10	2.87	0.18	3.06	2.15	1.40	2.20	0.71	1.81	0.05	1.15	0.00	1.46
Gentoo penguin	Crozet	Oct 2011	Blood	GPA11	1.26	0.10	1.36	1.98	1.30	2.18	0.60	1.72	0.04	1.21	-0.05	1.39
Macaroni penguin	Crozet	Jan 2012	Feathers	MPA11	1.96	0.40	2.16	2.88	1.93	3.10	0.96	2.37	0.00	1.65	-0.01	1.88
Macaroni penguin	Crozet	Jan 2012	Feathers	MPA12	1.88	0.41	2.08	3.02	1.97	3.05	0.98	2.34	0.08	1.57	-0.01	1.85

Macaroni penguin	Crozet	Jan 2012	Feathers	MPA13	1.98	0.63	2.20	3.15	2.26	3.26	1.05	2.51	-0.22	1.56	-0.08	1.94
Macaroni penguin	Crozet	Jan 2012	Feathers	MPA14	1.78	0.33	2.03	2.99	1.98	3.14	0.96	2.40	0.04	1.66	-0.03	1.91
Macaroni penguin	Crozet	Jan 2012	Feathers	MPA15	1.69	0.16	1.85	2.99	2.02	3.20	1.05	2.39	-0.03	1.68	0.04	1.89
Macaroni penguin	Crozet	Jan 2012	Feathers	MPA16	2.24	0.41	2.51	3.26	2.14	3.33	1.08	2.48	0.07	1.72	0.01	1.94
Macaroni penguin	Crozet	Jan 2012	Feathers	MPA17	2.21	0.64	2.43	3.07	2.10	3.09	1.09	2.39	-0.06	1.51	0.03	1.87
Macaroni penguin	Crozet	Jan 2012	Feathers	MPA18	2.20	0.27	2.39	3.09	2.10	3.11	0.98	2.39	-0.05	1.53	-0.08	1.86
Macaroni penguin	Crozet	Jan 2012	Feathers	MPA19	2.19	0.48	2.52	2.68	1.84	2.82	0.94	2.13	-0.07	1.44	0.01	1.66
Macaroni penguin	Crozet	Jan 2012	Feathers	MPA20	2.41	0.27	2.57	2.71	1.76	2.91	0.91	2.22	0.08	1.59	0.02	1.78
Macaroni penguin	Crozet	Jan 2012	Blood	MPA11	0.87	0.08	0.95	2.48	1.65	2.65	0.82	2.04	0.02	1.41	0.00	1.63
Macaroni penguin	Crozet	Jan 2012	Blood	MPA12	1.05	0.04	1.09	2.59	1.73	2.69	0.90	2.00	0.01	1.39	0.03	1.57
Macaroni penguin	Crozet	Jan 2012	Blood	MPA13	1.06	0.04	1.10	2.51	1.63	2.53	0.80	1.89	0.08	1.31	-0.02	1.48
Macaroni penguin	Crozet	Jan 2012	Blood	MPA14	0.80	0.02	0.82	2.49	1.67	2.64	0.86	2.05	0.00	1.38	0.02	1.63
Macaroni penguin	Crozet	Jan 2012	Blood	MPA15	0.91	0.04	0.95	2.31	1.60	2.52	0.81	1.94	-0.08	1.32	0.01	1.54
Macaroni penguin	Crozet	Jan 2012	Blood	MPA16	0.89	0.03	0.92	2.61	1.74	2.63	0.90	1.97	0.00	1.32	0.03	1.53
Macaroni penguin	Crozet	Jan 2012	Blood	MPA17	0.99	0.04	1.03	2.45	1.59	2.53	0.81	1.94	0.08	1.34	0.01	1.54
Macaroni penguin	Crozet	Jan 2012	Blood	MPA18	1.16	0.09	1.25	2.41	1.61	2.55	0.72	1.88	0.01	1.34	-0.09	1.48
Macaroni penguin	Crozet	Jan 2012	Blood	MPA19	1.13	0.04	1.18	2.23	1.49	2.40	0.71	1.84	0.01	1.28	-0.04	1.47
Macaroni penguin	Crozet	Jan 2012	Blood	MPA20	1.29	0.06	1.34	2.80	1.88	2.83	0.95	2.05	-0.01	1.41	0.00	1.57
Eastern rockhopper penguin	Crozet	Feb 2012	Feathers	SRPA11	1.41	0.08	1.48	3.31	2.18	3.44	1.06	2.59	0.05	1.80	-0.04	2.04
Eastern rockhopper penguin	Crozet	Feb 2012	Feathers	SRPA12	0.99	0.09	1.08	3.24	2.15	3.60	1.02	2.73	0.03	1.98	-0.06	2.19
Eastern rockhopper penguin	Crozet	Feb 2012	Feathers	SRPA13	1.14	0.08	1.22	3.54	2.36	3.67	1.22	2.69	0.02	1.89	0.03	2.10
Eastern rockhopper penguin	Crozet	Feb 2012	Feathers	SRPA14	1.45	0.14	1.59	3.44	2.27	3.49	1.14	2.66	0.05	1.78	0.00	2.08
Eastern rockhopper penguin	Crozet	Feb 2012	Feathers	SRPA15	1.47	0.15	1.62	3.31	2.17	3.28	1.11	2.52	0.07	1.65	0.03	1.98
Eastern rockhopper penguin	Crozet	Feb 2012	Feathers	SRPA16	1.52	0.17	1.69	3.73	2.51	3.64	1.26	2.58	-0.02	1.75	0.00	1.95
Eastern rockhopper penguin	Crozet	Feb 2012	Feathers	SRPA17	1.48	0.14	1.62	3.67	2.41	3.64	1.26	2.71	0.07	1.83	0.04	2.10

Eastern rockhopper penguin	Crozet	Feb 2012	Feathers	SRPA18	0.98	0.08	1.06	3.77	2.45	3.74	1.34	2.82	0.12	1.89	0.11	2.20
Eastern rockhopper penguin	Crozet	Feb 2012	Feathers	SRPA19	1.25	0.11	1.36	3.50	2.34	3.53	1.15	2.67	0.01	1.78	-0.02	2.08
Eastern rockhopper penguin	Crozet	Feb 2012	Feathers	SRPA20	1.10	0.07	1.17	3.36	2.25	3.61	1.14	2.75	0.00	1.91	0.01	2.18
Eastern rockhopper penguin	Crozet	Feb 2012	Blood	SRPA11	0.80	0.05	0.84	2.57	1.67	2.72	0.89	2.13	0.07	1.46	0.05	1.71
Eastern rockhopper penguin	Crozet	Feb 2012	Blood	SRPA12	0.74	0.04	0.78	2.92	2.00	3.13	1.00	2.45	-0.06	1.63	0.00	1.95
Eastern rockhopper penguin	Crozet	Feb 2012	Blood	SRPA13	1.04	0.08	1.12	3.04	2.00	3.06	1.05	2.27	0.06	1.56	0.05	1.77
Eastern rockhopper penguin	Crozet	Feb 2012	Blood	SRPA14	1.07	0.05	1.13	3.00	2.01	3.08	1.05	2.34	-0.01	1.57	0.04	1.83
Eastern rockhopper penguin	Crozet	Feb 2012	Blood	SRPA15	0.89	0.05	0.93	2.74	1.89	2.99	0.96	2.35	-0.08	1.57	0.01	1.87
Eastern rockhopper penguin	Crozet	Feb 2012	Blood	SRPA16	1.34	0.05	1.39	3.22	2.12	3.05	1.08	2.18	0.06	1.46	0.01	1.65
Eastern rockhopper penguin	Crozet	Feb 2012	Blood	SRPA17	1.00	0.03	1.03	3.04	2.05	3.09	1.03	2.26	-0.02	1.54	0.00	1.74
Eastern rockhopper penguin	Crozet	Feb 2012	Blood	SRPA18	0.87	0.03	0.89	3.10	2.12	3.05	0.93	2.09	-0.07	1.45	-0.13	1.55
Eastern rockhopper penguin	Crozet	Feb 2012	Blood	SRPA19	0.78	0.03	0.81	2.78	1.81	2.79	0.89	2.12	0.08	1.42	-0.02	1.67
Eastern rockhopper penguin	Crozet	Feb 2012	Blood	SRPA20	0.73	0.04	0.77	2.45	1.61	2.90	0.93	2.32	0.05	1.69	0.12	1.91

Table S2. Mean values of Hg isotopic composition obtained for reference materials with validated reference isotopic values: NIST RM 8610 (former UM-Almadén), NIST SRM 1947 (Lake Michigan fish tissue) and NIES-13 (human hair); previously published intercomparison values: IAEA-086 (human hair), ERM-CE-464 (tuna fish); and internal reference materials: F-KP (penguin feathers) and RBC-KP (penguin red blood cells).

Sample	Reference	n	$\delta^{204}\text{Hg}\pm 2\text{SD}$ ‰	$\delta^{202}\text{Hg}\pm 2\text{SD}$ ‰	$\delta^{201}\text{Hg}\pm 2\text{SD}$ ‰	$\delta^{200}\text{Hg}\pm 2\text{SD}$ ‰	$\delta^{199}\text{Hg}\pm 2\text{SD}$ ‰	$\Delta^{204}\text{Hg}\pm 2\text{SD}$ ‰	$\Delta^{201}\text{Hg}\pm 2\text{SD}$ ‰	$\Delta^{200}\text{Hg}\pm 2\text{SD}$ ‰	$\Delta^{199}\text{Hg}\pm 2\text{SD}$ ‰
	This study	77	-0.84±0.22	-0.55±0.16	-0.45±0.13	-0.27±0.51	-0.17±0.12	-0.01±0.16	-0.04±0.09	0.00±0.11	-0.03±0.11

NIST RM 8610 (UM Almadén)	Reference values		-0.82±0.07	-0.56±0.03	-0.46±0.02	-0.27±0.01	-0.17±0.01	-	-0.04±0.01	0.00±0.01	-0.03±0.02
NIST SRM 1947	This study	4	1.68±0.25	1.19±0.10	5.02±0.15	0.69±0.09	5.54±0.14	-0.09±0.12	4.13±0.12	0.09±0.05	5.25±0.13
	Reference values		1.66±0.07	1.20±0.07	5.09±0.18	0.69±0.09	5.62±0.25	-0.13±0.02	4.17±0.28	0.09±0.02	5.31±0.29
NIES-13	This study	8	2.99±0.22	2.08±0.15	3.07±0.16	1.09±0.10	2.34±0.10	-0.12±0.28	1.51±0.06	0.04±0.04	1.81±0.09
	Yamakawa et al., 2016	11	2.76±0.16	1.89±0.10	2.77±0.10	0.98±0.08	2.13±0.07	-0.04±0.11	1.36±0.07	0.04±0.04	1.65±0.06
IAEA-086	This study	6	0.94±0.25	0.73±0.13	0.77±0.09	0.37±0.11	0.44±0.16	-0.14±0.26	0.22±0.08	0.00±0.09	0.26±0.15
	Yamakawa et al., 2016	3	0.87±0.12	0.58±0.09	0.64±0.09	0.31±0.04	0.41±0.02	0.00±0.04	0.20±0.03	0.02±0.04	0.26±0.02
ERM-CE-464	This study	2	0.91±0.13	0.64±0.04	2.37±0.03	0.40±0.01	2.49±0.01	-0.04±0.19	1.89±0.05	0.08±0.01	2.33±0.01
	Li et al. , 2014	9	0.94±0.06	0.70±0.04	2.49	0.43±0.05	2.55±0.08	-	1.96±0.06	0.07±0.04	2.38±0.07
F-KP	This study	17	2.40±0.25	1.58±0.22	2.67±0.21	0.80±0.16	2.11±0.18	0.04±0.24	1.48±0.13	0.01±0.09	1.71±0.15
RBC-KP	This study	7	1.98±0.18	1.30±0.13	2.26±0.14	0.64±0.13	1.78±0.17	0.04±0.10	1.28±0.09	-0.01±0.09	1.46±0.14

Table S3. Internal reproducibility of Hg isotopic composition obtained for internal reference materials: F-KP (penguin feathers) and RBC-KP (penguin red blood cells) during the performed analytical sessions. N means number of analysis for the same sample extracts, which were prepared under similar procedures for each session.

Sample	Analytical session												
		$\delta^{204}\text{Hg}$	$\delta^{202}\text{Hg}$	$\delta^{201}\text{Hg}$	$\delta^{200}\text{Hg}$	$\delta^{199}\text{Hg}$	$\Delta^{204}\text{Hg}$	$\Delta^{201}\text{Hg}$	$\Delta^{200}\text{Hg}$	$\Delta^{199}\text{Hg}$	n		
King penguin feathers	F-KP	May 2015	Average (‰)	2.30	1.52	2.64	0.77	2.11	0.09	1.50	0.00	1.73	8
			2SD (‰)	0.32	0.22	0.20	0.11	0.08	0.16	0.06	0.06	0.08	

		March 2016	Average (‰)	2.40	1.58	2.68	0.80	2.11	0.04	1.49	0.00	1.71	10
			2SD (‰)	0.24	0.26	0.17	0.15	0.18	0.31	0.12	0.06	0.15	
		July 2016	Average (‰)	2.44	1.60	2.70	0.85	2.15	0.05	1.50	0.04	1.74	5
			2SD (‰)	0.26	0.15	0.16	0.18	0.15	0.11	0.07	0.12	0.12	
King penguin blood	RBC-KP	March 2016	Average (‰)	1.95	1.32	2.28	0.65	1.80	-0.01	1.28	-0.01	1.47	3
			2SD (‰)	0.10	0.10	0.04	0.03	0.04	0.15	0.11	0.03	0.02	
		April 2016	Average (‰)	1.60	0.99	2.08	0.52	1.84	0.12	1.34	0.02	1.59	3
			2SD (‰)	0.37	0.20	0.29	0.36	0.08	0.15	0.14	0.25	0.05	
		July 2016	Average (‰)	1.99	1.30	2.26	0.62	1.77	0.05	1.28	-0.03	1.44	5
			2SD (‰)	0.15	0.10	0.12	0.11	0.16	0.10	0.10	0.07	0.15	

Total Hg and Hg species concentrations

Pronounced differences in total Hg concentrations were found between sites for both skua and penguin populations. However, no variation in Hg species distribution was observed between tissues, individuals, species or sites. Both feathers and blood presented a majority of MeHg for all the individuals (skuas and penguins) independently of Hg concentrations. Percentages of MeHg mean values for feathers and blood of the four skua populations were $94\pm 2\%$ and $94\pm 3\%$, respectively. Overall penguins' individuals presented a mean percentage of MeHg of $92\pm 2\%$ and $92\pm 7\%$ respectively for feathers and blood samples. Skua chick's feathers displayed higher MeHg concentrations ($\mu\text{g/g}$ dry mass) compared to blood samples for all the analysed individuals (3 times higher, on average for the four populations). In the case of penguins, similar concentrations were obtained in both tissues for some penguin species (Adélie, king and both eastern and northern rockhopper penguins), whereas feathers presented higher MeHg levels than blood in all the individuals of gentoo and macaroni penguins (2 times higher on average for the two populations). As a general trend, blood and feathers MeHg concentrations were highly correlated for skuas (Pearson correlation, $R^2=0.89$, $p<0.0001$, $n=40$) and penguin individuals (Pearson correlation, $R^2=0.74$, $p<0.0001$, $n=72$), as shown in SI (Figures S2 and S3).

Figure S2. Feather versus blood (red blood cells) MeHg concentrations in skua chicks. Regression equations for MeHg blood-feather values is $y=2.53x+1.05$ ($R^2=0.79$, $p<0.0001$).

Abbreviations for the different populations are AL (Adélie Land), Ker (Kerguelen), Cro (Crozet) and Ams (Amsterdam).

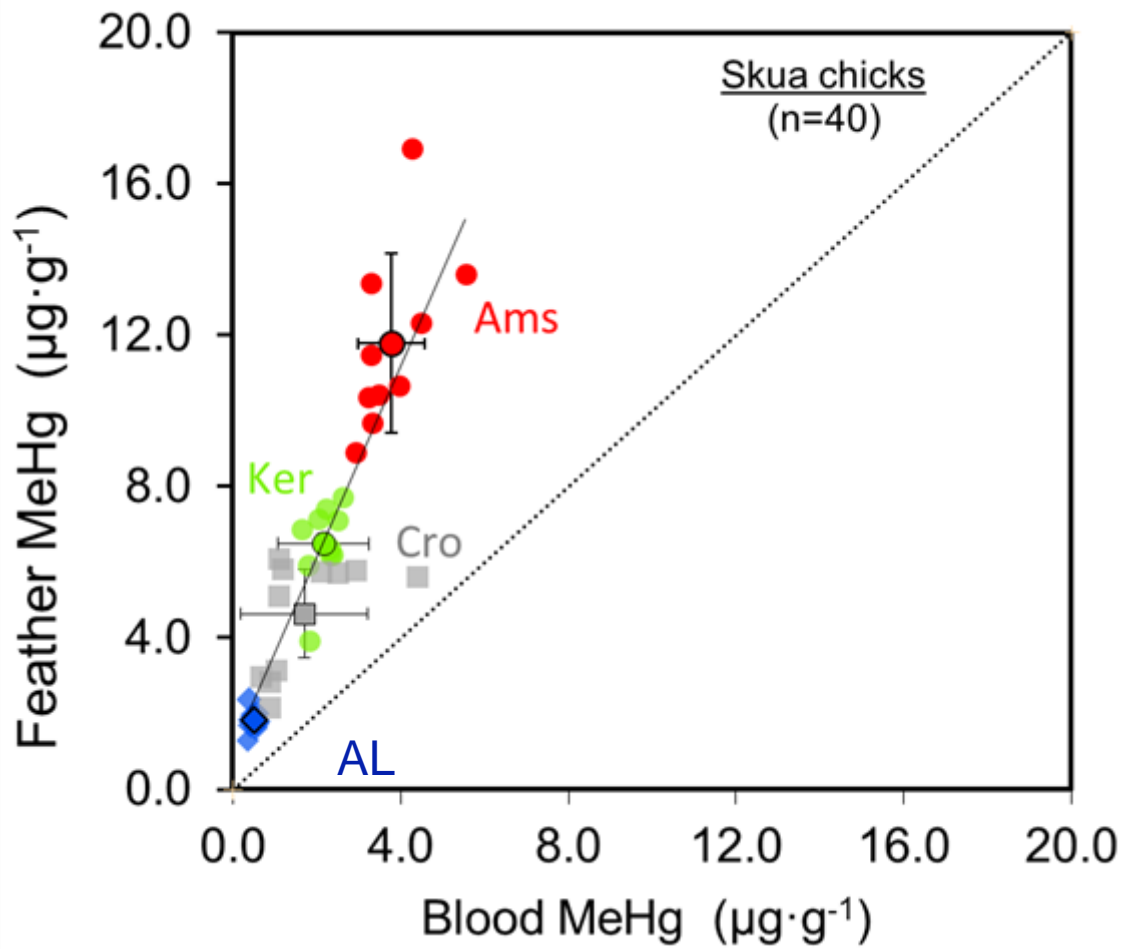


Figure S3. Feather versus blood (red blood cells) MeHg concentrations in adult penguins (individual values and mean population values). Regression equations for MeHg blood-feather values is $y=1.28x+0.50$ ($R^2=0.50$, $p<0.0001$). Abbreviations for the different populations are AP (Adélie penguin), GP (gentoo penguin), KP (king penguin), MP (macaroni penguin), RP (eastern rockhopper penguin) and NRP (northern rockhopper penguin).

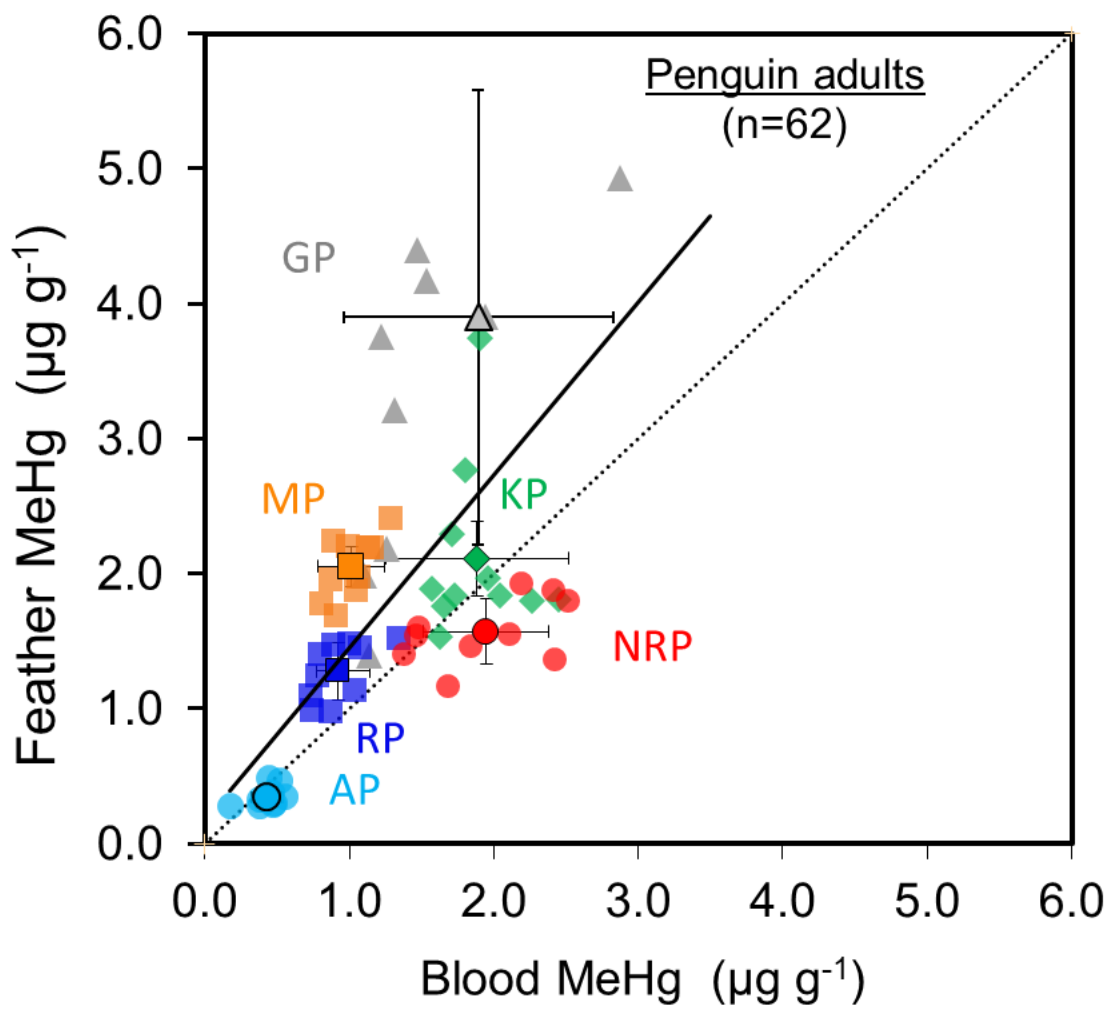


Figure S4. Blood and feathers $\delta^{202}\text{Hg}$ values for the four populations of skua chicks. Diamonds indicate mean values. Black lines indicate median values. Red asterisks indicate outliers.

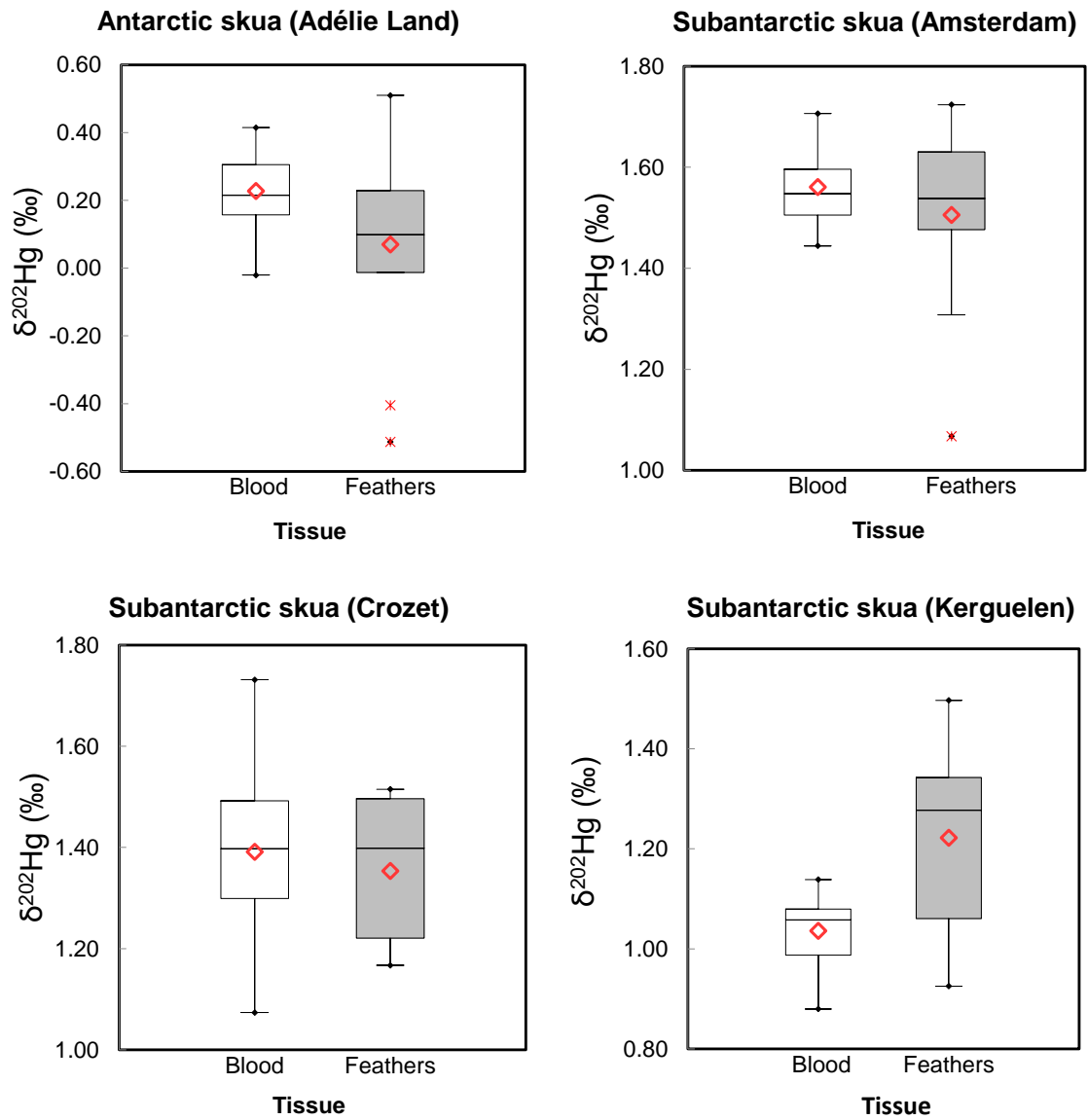


Figure S5. Blood and feathers $\Delta^{199}\text{Hg}$ values for the four populations of skua chicks. Diamonds indicate mean values. Black lines indicate median values. Red asterisk indicates outlier.

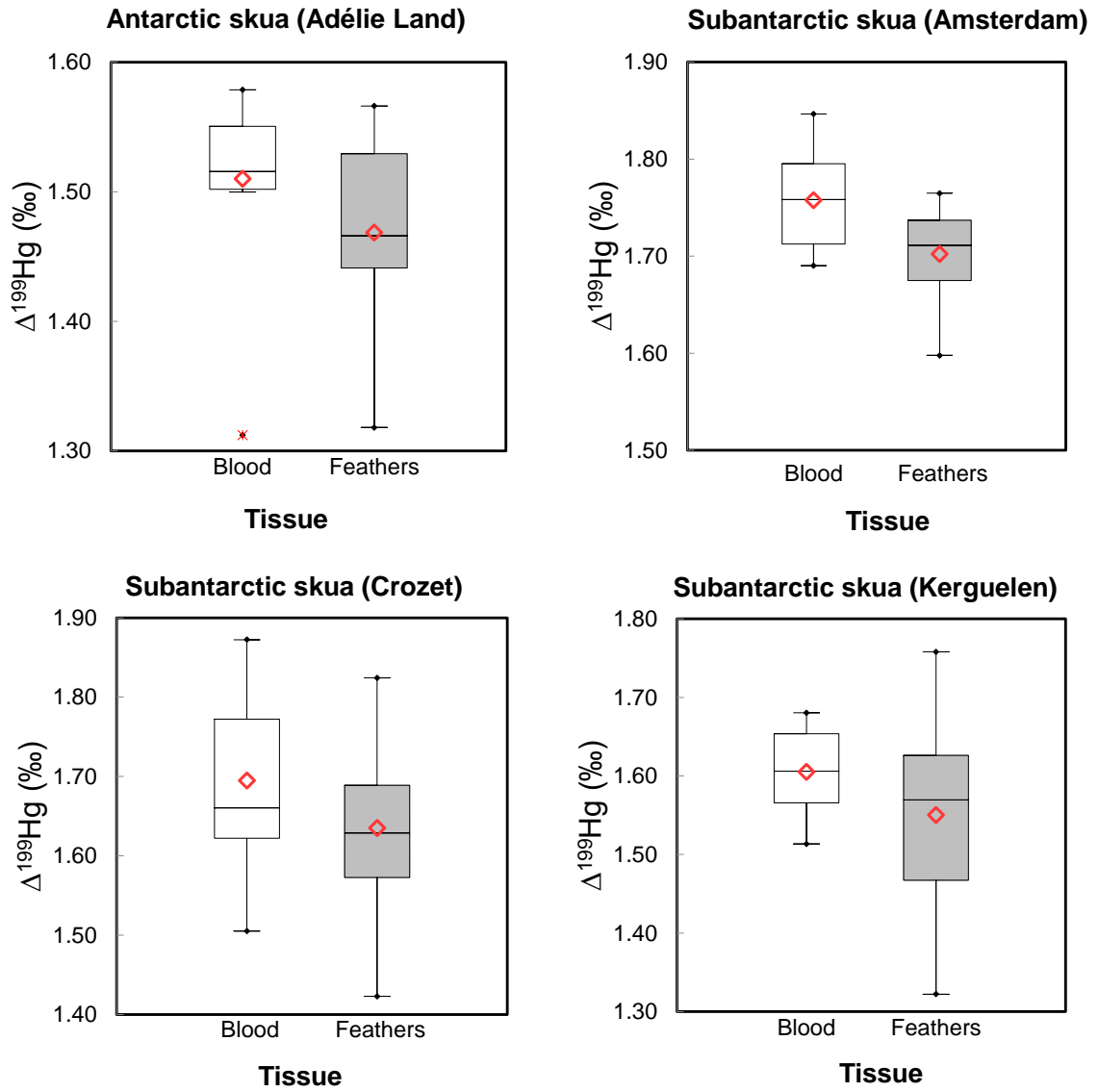


Figure S6. Blood and feathers $\delta^{202}\text{Hg}$ values for the six populations of penguin adults. Diamonds indicate mean values. Black lines indicate median values. Red asterisks indicate outliers.

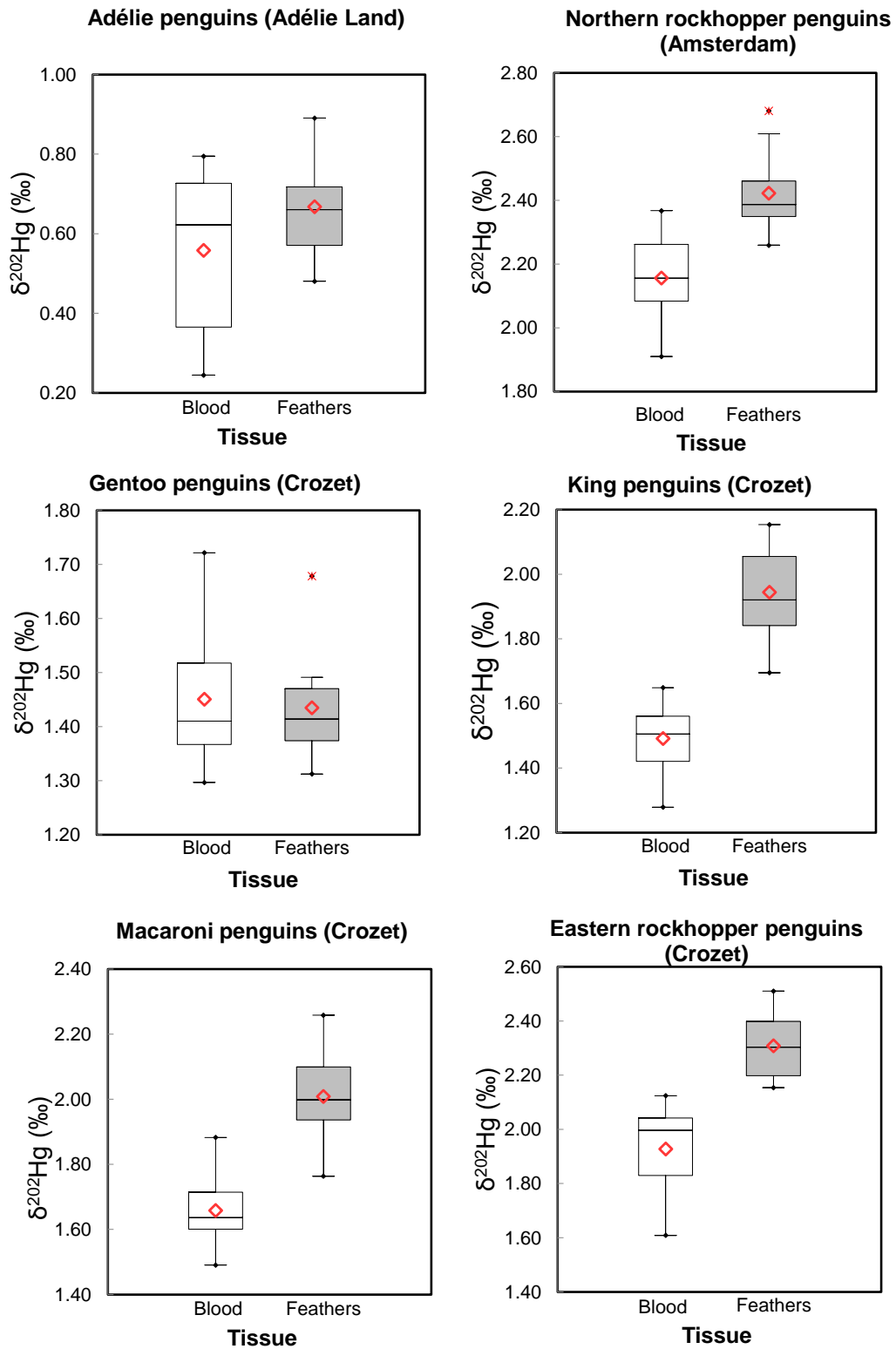
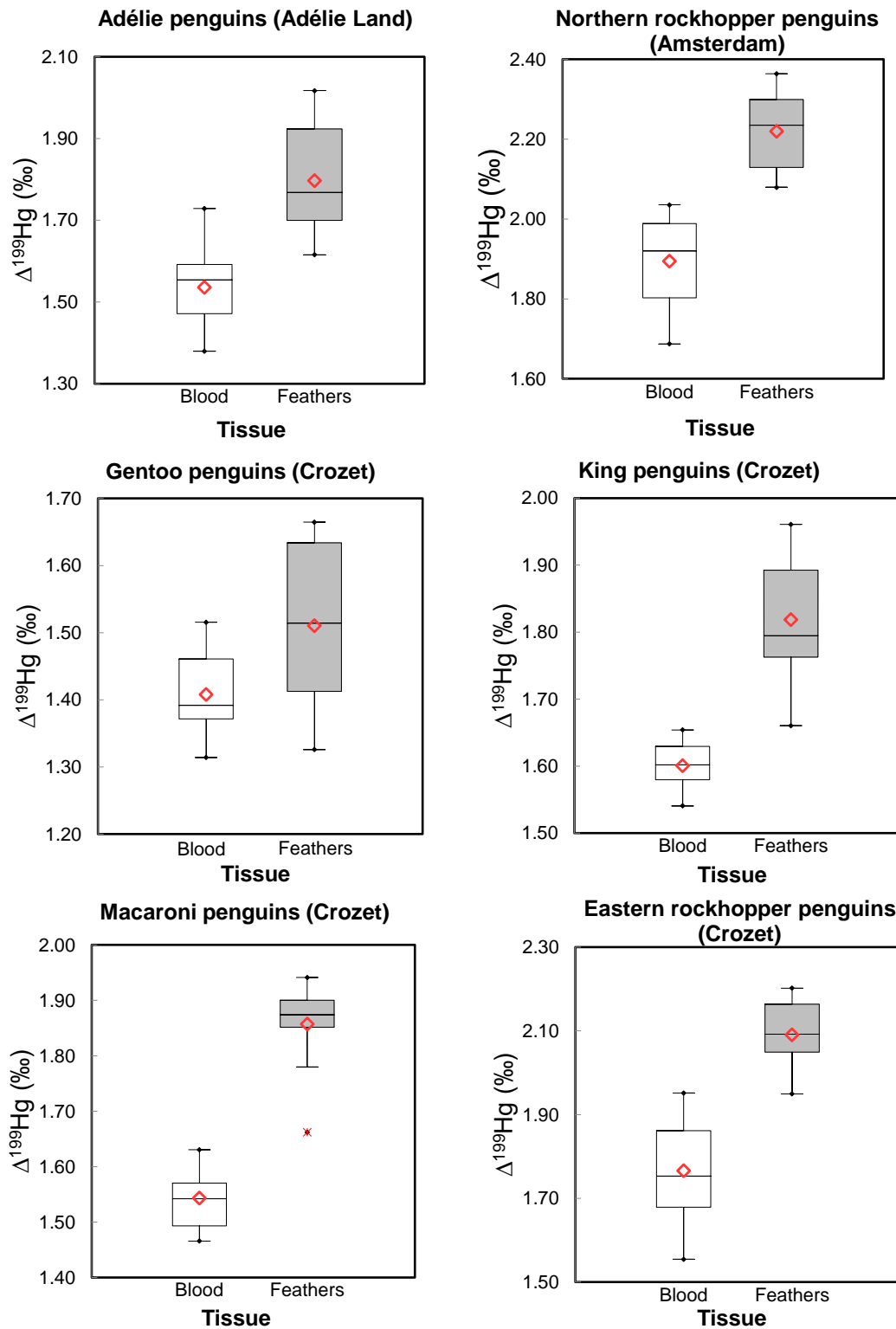


Figure S7. Blood and feathers $\Delta^{199}\text{Hg}$ values for the six populations of penguin adults. Diamonds indicate mean values. Black lines indicate median values. Red asterisk indicates outliers.



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