Circumpolar assessment of mercury contamination: the Adélie penguin as a bioindicator of Antarctic marine ecosystems

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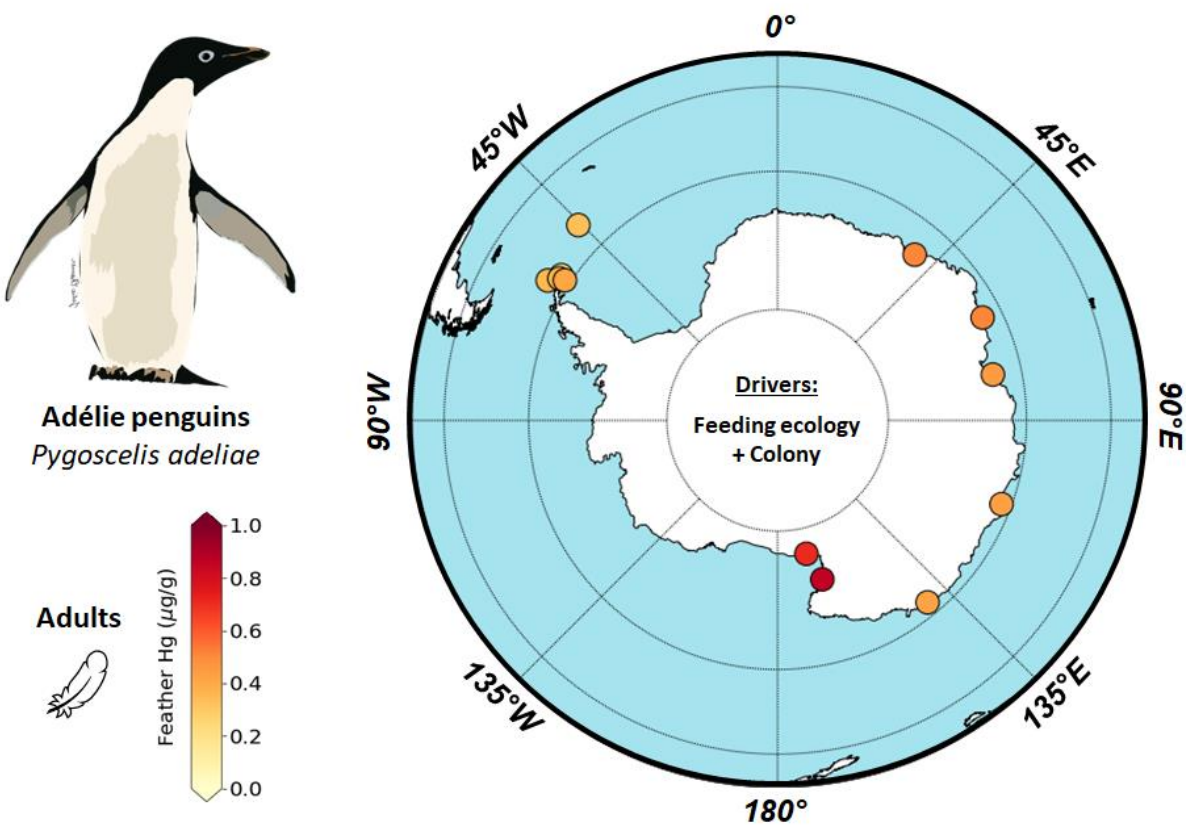
HIGHLIGHTS

- Adélie penguins are relevant bioindicators of Hg contamination in Antarctic marine ecosystems
- Feather Hg concentrations were measured in 24 breeding colonies (adults and chicks)
- The highest Hg concentrations were found in the Ross Sea
- Both trophic ecology and colony location drove feather Hg concentrations

GRAPHICAL ABSTRACT



ABSTRACT: Due to its persistence and potential ecological and health impacts, mercury (Hg) is a global pollutant of major concern that may reach high concentrations even in remote polar oceans. In contrast to the Arctic Ocean, studies documenting Hg contamination in the Southern Ocean are spatially restricted and large-scale monitoring is needed. Here, we present the first circumpolar assessment of Hg contamination across Antarctic marine ecosystems. Specifically, the Adélie penguin (*Pygoscelis adeliae)* was used as a bioindicator species, to examine regional variation across 24 colonies distributed across the entire Antarctic continent. Mercury was measured on body feathers collected from both adults (n=485) and chicks (n=48) between 2005 and 2021. Because penguins' diet represents the dominant source of Hg, feather $\delta^{13}C$ and $\delta^{15}N$ values were measured as proxies of feeding habitat and trophic position. As expected, chicks had lower Hg concentrations (mean ± SD: 0.22 ± 0.08 $\mu g \cdot g^{-1}$) than adults (0.49 ± 0.23 $\mu g \cdot g^{-1}$), likely because of their shorter bioaccumulation period. In adults, spatial variation in feather Hg concentrations was driven by both feeding ecology and colony location. The highest Hg concentrations were observed in the Ross Sea, possibly because of a higher consumption of fish in the diet compared to other sites (krill-dominated diet). Such large-scale assessments are critical to assess the effectiveness of the Minamata Convention on Mercury. Owing to their circumpolar distribution and their ecological role in Antarctic marine ecosystems, Adélie penguins could be valuable bioindicators for tracking spatial and temporal trends of Hg across Antarctic waters in the future.

KEYWORDS

Feathers – Hg – Marine food web – Seabirds – Stable Isotopes – Southern Ocean

INTRODUCTION

Mercury (Hg) is a global pollutant of both natural and anthropogenic origin (Sonke et al., 2023). Since the Industrial Revolution, anthropogenic activities, such as chemical manufacturing, gold-mining and coal combustion (Streets et al., 2017), have released considerable amounts of Hg into the environment, resulting in a three- to five-fold increase of Hg globally (Lamborg et al., 2014; Selin, 2009). Yet, this non-essential and toxic metal is of global concern due to its adverse effects on wildlife and human health (NRC/NAS, 2000; Ackerman et al., 2016; Dietz et al., 2019; Roman et al., 2011; Scheuhammer et al., 2012; Tan et al., 2009). As a reaction, the Minamata Convention on Hg was adopted in 2013 (implemented in 2017) by more than140 countries, to protect human health and the environment worldwide. Today, large-scale monitoring of environmental Hg contamination is required to assess the effectiveness of this international treaty and guide its future directions.

Mercury disperses worldwide mainly through atmospheric currents and deposits even in the most remote oceanic regions, such as polar oceans (UN-Environment, 2019). These oceans are facing significant modifications due to climate change, which alters the biogeochemical cycle of Hg (Chételat et al., 2022; McKinney et al., 2022) and ultimately its transfer in marine food webs (Zhou et al., 2023). In the Arctic Ocean, Hg contamination has been extensively documented over large temporal and spatial scales (*e.g.*, Albert et al., 2021; Bond et al., 2015; Desforges et al., 2022; Dietz et al., 2022). In the Southern Ocean (*sensu lato, i.e.*, water masses south of the Subtropical Front; Carter et al., 2008; Orsi et al., 1995), studies are more spatially restricted mainly due to logistical constraints, with a focus on local and/or regional scales (but see Brasso et al., 2015; Carravieri et al., 2017; Cherel et al., 2018). This is particularly the case in the Antarctic Zone (*i.e.*, water masses south of the Polar Front; Carter et al., 2008; Orsi et al., 1995). This critical gap in sampling areas can be filled by using bioindicator species that closely rely on marine food webs from these specific areas, such as seabirds (*e.g.*, Burger and Gochfeld, 2004; Furness and Camphuysen, 1997). As meso- to top predators, seabirds are abundant consumers of the world's marine resources. Due to biomagnification processes, they reflect Hg contamination of their marine food webs (Braune et al., 2015; Fort et al., 2016; Piatt et al., 2007). Food intake is the major source of Hg in seabirds, and thus assessing their feeding ecology and spatial distribution is needed to disentangle ecological drivers of Hg contamination (Carravieri et al., 2014b; Cherel et al., 2018). Specifically, accounting for feeding ecology is key to identify whether spatial variation in Hg contamination is linked to dietary differences (Brasso et al., 2015; Carravieri et al., 2014c; Gatt et al., 2020) and/or to other environmental factors (Foster et al., 2019; Furtado et al., 2021; Tartu et al., 2022).

Once assimilated by seabirds, Hg is distributed *via* blood to internal tissues where it bioaccumulates (*i.e.*, Hg concentrations increase over time) between moulting periods. During moult, stored Hg is remobilised and up to 90% of the total Hg body burden is excreted into growing feathers (Honda et al., 1986), where it binds to keratin proteins (Crewther et al., 1965). Hence, moult represents the main excretion pathway for Hg in seabirds (Thompson et al., 1990; Thompson and Furness, 1989). Feathers incorporate the cumulative signal of Hg exposure between two moulting episodes (*i.e*., over several weeks/months to up to one year in most species; Albert et al., 2019). Sampling seabird feathers thus represent a powerful monitoring opportunity to investigate Hg contamination in marine food webs over large temporal and spatial scales, especially in the remote Southern Ocean.

Penguins constitute the largest seabird biomass in the Southern Ocean and consume several million tons of marine resources annually (Knox, 2006; Southwell et al., 2017; Williams, 1995). Available evidence suggests that they exploit similar marine resources over both the short-term (breeding season) and the long-term (non-breeding season; Cherel et al., 2007; Polito et al., 2016; Tierney et al., 2009). Therefore, penguins are adequate bioindicator species to monitor Hg contamination in Antarctic marine food webs (Brasso et al., 2015; Carravieri et al., 2013). Among seabirds, penguins present a unique moulting pattern. Following a pre-moult foraging period of hyperphagia at sea, they renew their entire plumage annually while fasting ashore or on sea ice (a few weeks; Cherel et al., 1994; Emmerson et al., 2019), less frequently at the breeding colonies (Ainley, 2002). All body feathers are thus moulted simultaneously every year. Within each individual penguin, all body feathers have thus the same chemical signature, including Hg concentrations and stable isotope ratios (Brasso et al., 2013; Carravieri et al., 2014a). Since the 1980s, 20 studies documented Hg contamination in feathers of eight penguin species from the Southern Ocean, including species breeding both in the Subantarctic (*i.e.*, water masses between the Subtropical and Polar Fronts) and Antarctic Zones (see Table S1 for a complete review). Whereas contamination from subantarctic islands has been well documented, spatial coverage is limited to local and regional investigations in the Antarctic. For example, out of the 14 Hg studies in the Antarctic Zone, nine (64%) were carried out in the South Shetland Islands only (e.g. Álvarez-Varas et al., 2018; Becker et al., 2016; Brasso et al., 2015; Matias et al., 2022; Souza et al., 2020). Other documented regions include the coasts of the Queen Maud Land, Adélie Land and Victoria Land (Ross Sea) (Bargagli et al., 1998; Carravieri et al., 2016; Pilcher et al., 2020; Yamamoto et al., 1996). To date, most Antarctic regions where penguins breed remain unexplored. Large-scale sampling of penguin feathers is thus needed to identify potential hotspots of Hg contamination and determine the toxicological risk to Antarctic marine biodiversity, which is simultaneously threatened by other anthropogenic stressors, including climate change (Barbraud et al., 2012; Clucas et al., 2014; Lee et al., 2017; Morley et al., 2019).

The Adélie penguin (*Pygoscelis adeliae*) is an ideal candidate to reflect Hg contamination in Antarctic marine food webs. With its circumpolar distribution, it is the most common and abundant penguin species in both continental and maritime (Antarctic Peninsula and adjacent archipelagos) Antarctica. Adélie penguins forage in Antarctic waters year-round (Ballard et al., 2010; Takahashi et al., 2018; Thiebot et al., 2019) and consistently use similar feeding resources (Ainley, 2002; Jafari et al., 2021; Juáres et al., 2016). The Adélie penguin is an indicator species for the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) for the Ecosystem Monitoring Program (Agnew, 1997). This latter aims (i) to ensure that krill fisheries take account of the needs of krill-predators, such as seals and seabirds, and (ii) to distinguish between environmental variation/changes and fishing activities in the Southern Ocean. Here, we take advantage of a large, international field-based scientific network, to provide the first circumpolar assessment of Hg contamination in Antarctic marine ecosystems, using feathers of Adélie penguins breeding across both continental and maritime Antarctica. The aims of this study were four-fold. The first aim was to quantify current Hg contamination of Antarctic marine food webs by focussing on this key higher-order indicator species at a circumpolar scale. Given the unique oceanographic features of the Southern Ocean (*i.e.*, circumpolar circulation), relatively homogenous Hg concentrations were expected across all Adélie penguin colonies. The second aim was to identify potential Hg hotspots by assessing Hg contamination over different spatial and temporal scales, using fledging-chick feathers, which reflect short-term, local contamination (~two months during the breeding season only), and adult feathers, which integrate a geographically larger scale over one year, incorporating both the breeding and non-breeding seasons. Because of longer exposure to Hg, adults were expected to show higher Hg concentrations than chicks (Stewart et al., 1997; Thompson et al., 1991). The third aim was to investigate the influence of penguin trophic ecology on spatial patterns of Hg contamination, by using feather carbon- ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes, although Hg and stable isotopes are decoupled in feathers (Bond, 2010). Stable isotopes are well-known proxies of feeding habitat and trophic position, respectively (Kelly, 2000; Newsome et al., 2007) and still provide relevant information on feeding ecology for the understanding of Hg contamination. As for other species and regions, trophic ecology was expected to be a major driver of spatial differences of Hg contamination in Antarctic marine ecosystems (*e.g.*, Becker et al., 2002; Bustamante et al., 2016; Carravieri et al., 2014c; Mills et al., 2020). Finally, the last aim was to investigate sex differences in both Hg contamination and trophic ecology using a subset of adult Adélie penguins from various colonies.

MATERIAL AND METHODS

Feather collection and preparation

This comprehensive assessment brings together published and new Hg concentrations for Adélie penguin feathers (Table 1 and 2). Overall, 538 individuals of Adélie penguins (490 breeding adults and 48 pre-fledging chicks) were sampled between 2005 and 2021, in 24 colonies around Antarctica (n=5−40 individuals per colony; Fig.1). Sample sizes for each colony are provided in Tables 1 (adults) and 2 (chicks) and geographical coordinates of sampled colonies are detailed in the Supplementary Material (Table S2). For both adults and chicks, body feathers were sampled for two reasons: (i) they are generally considered to be the best feather type to sample (Furness et al., 1986), and (ii) in penguins, they represent the majority of the plumage (no flight feather). Therefore, 1−5 body feathers from the breast or the back were collected for each individual, because of their complete and drastic moulting strategy (Brasso et al., 2013; Cherel et al., 1994). In a limited number of colonies, where no other pygoscelid penguin breeds, freshly molted feathers were also collected from the ground (*i.e*., in Shirley Island, Brown Bluffs, Madder Cliffs and Paulet Island; feather sampling locations in each of these colonies were at least several nests away from each other to avoid pseudoreplication; minimum distance = 15 m). Feathers were then stored in either plastic bags or paper envelopes and kept at room temperature until laboratory analyses.

In adults, feather moult starts when the penguins are still at sea, building body reserves during the pre-moulting period (Cherel et al., 1993, 1994; Croxall, 1982). The part of the feather that grows at this time (*i.e.*, the tip) thus reflects the pre-moult feeding period at sea. In contrast, the remaining feather that grows during moult reflects the fasting period on sea ice or ashore, and hence the dietary signature from accumulated body reserves. Given the difference in integration of dietary information (stable isotopes) during these two periods, feather's tip (*i.e.*, 5 mm) was cut and discarded. Both Hg and stable isotopes were analysed in the remaining feather. In order to avoid any external contamination, feathers were cleaned with a chloroform:methanol mixture (2:1), sonicated for 3 min, rinsed twice with methanol and dried at 45°C for 48 h. Given the homogenous chemical composition of body feathers in penguins

(Brasso et al., 2013; Carravieri et al., 2014a), measurements were made on a single randomly-selected feather for each bird, which was cut with precision scissors to obtain a homogenous powder to be analysed for both Hg and stable isotopes.

Mercury analyses

Total Hg (THg) includes both inorganic and organic Hg (mainly methyl-Hg, MeHg). In feathers, >90% of THg is in the form of MeHg (Renedo et al., 2017), the most toxic and bioavailable form that bioaccumulates and biomagnifies in marine food webs (Cossa, 2013). Therefore, we used THg as a proxy of MeHg in Adélie penguin feathers.

Mercury analyses were performed on feather homogenates (0.5–1.5 mg) in duplicate, using an Advanced Mercury Analyser (AMA 254, Altech). When the relative standard deviation (RSD) between duplicates was <10%, Hg concentrations were averaged for each sample. When the RSD was >10%, an additional sample of the homogenate was analysed, and the duplicates guaranteeing the lowest RSD were kept for average calculations. The AMA quantification limit was 0.1 ng. Blanks and two certified reference materials (TORT3 – Lobster hepatopancreas, DOLT5 – Fish liver, NRC Canada) were analysed during each analytical session to guarantee accuracy (Table S3). Recovery values were 100.6 ± 2.3% and 96.7 ± 2.6%, respectively. Concentrations are expressed as $\mu g \cdot g^{-1}$ dry weight (dw).

Stable isotope analyses

Carbon and nitrogen stable isotope analyses were performed on feather homogenates (0.2–0.8 mg), loaded into tin cups (8 mm x 5 mm; Elemental Microanalysis Ltd, Okehampton, UK) using a microbalance (XPRUD5, Mettler Toledo, Greifensee, Switzerland). Values of $\delta^{13}C$ and $\delta^{15}N$ were determined with a continuous flow isotope ratio mass spectrometer (Delta V Plus with a Conflo IV Interface, Thermo Scientific, Bremen, Germany) coupled to an elemental analyzer (Flash 2000 or Flash IRMS EA Isolink CN, Thermo Scientific, Milan, Italy). Results are expressed in the usual δ unit notation relative to Vienna PeeDee Belemnite for $\delta^{13}C$ (‰) and atmospheric N_2 for $\delta^{15}N$ (‰), following the formula:

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 10^3$$

where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively. Replicate measurements of reference materials (USGS61 and USGS63, US Geological Survey) indicated measurement uncertainties <0.10 ‰ for both $\delta^{13}C$ and $\delta^{15}N$ values. Further methodological details are provided in the Supplementary Material.

Combining feather Hg and stable isotopes

While Hg and stable isotopes are both incorporated during feather growth, their integration period is temporally decoupled in adult seabirds (Bond, 2010). Feathers indicate Hg accumulation in the whole body since the previous moult (*i.e.*, integration period: one year in adult penguins), a temporal period that includes different stages of their life cycle (*i.e.*, non-breeding, migration/return to colony, breeding, post-breeding dispersal and moult). In contrast, stable isotopes represent the diet during feather synthesis (*i.e*., integration period: a few weeks corresponding to the pre-moult foraging period). However, available evidence suggests that Adélie penguins consistently use similar feeding resources during the non-breeding period (Polito et al., 2016; Tierney et al., 2009). Thus, stable isotopes are still relevant to understand the ecological drivers of Hg contamination in adult Adélie penguins. In large fledging chicks, there is no such temporal mismatch between Hg concentrations and stable isotopes, as both are incorporated during the growth of body feathers and reflect local contamination and diet during the same period (chick-rearing period; ~two months in summer).

Statistical analyses

Data analyses and representation («ggplot2» package; Wickham, 2016) were carried out with R (Version 4.2.2, R Core Team 2022).

During data exploration, five feather samples showed extremely high $\delta^{13}C$ values, which are theoretically associated with either a coastal/benthic or a subtropical environment instead of offshore/pelagic Antarctic $\delta^{13}C$ values, as expected (Carravieri et al., 2014b; Cherel and Hobson, 2007). Feather Hg and stable isotope values for these five individuals are presented in Supplementary Material (Table S4). Following a conservative approach, these values were considered as outliers and were therefore excluded from statistical analyses and figures.

Spatial variation in Hg contamination and trophic ecology

Unifactorial analyses were performed to investigate independently differences in feather Hg concentrations and $\delta^{13}C$ and $\delta^{15}N$ values between colonies. Residual normality and homoscedasticity were examined with Shapiro-Wilk and Breusch-Pagan tests («lmtest» package; Zeileis and Hothorn, 2002), respectively. Since test assumptions were not met, a non-parametric (Kruskal-Wallis) test was used, followed by a multiple comparisons (Pairwise-Wilcoxon) test.

Drivers of Hg contamination and its spatial variation

Multifactorial analyses were performed to investigate simultaneously the influence of trophic ecology, colony location, sex and age class on Hg contamination. Prior to model definition, relationships between continuous variables (*i.e.*, Hg, $\delta^{13}C$, $\delta^{15}N$) were tested using a correlation matrix to validate the simultaneous inclusion of non-collinear explanatory variables. Models were Generalized Linear Models (GLMs) with a Gaussian distribution and either identity or inverse link-functions, built using the «nlme» R package (Pinheiro and Bates, 2022).

We used three datasets: adults only (n=485), adults of known sex (n=231) and adults and pre-fledging chicks sampled at the same colony (n=113), to test for different effects with the larger available sample size. Initial models for each dataset were the following: (1) Hg ~ $\delta^{13}C$ + $\delta^{15}N$ + Colony, (2) Hg ~ $\delta^{13}C$ + $\delta^{15}N$ + Colony * Sex and (3) Hg ~ $\delta^{13}C$ + $\delta^{15}N$ + Colony * Age class.

Model selection was based on Akaike's Information Criterion adjusted for small sample sizes (AIC_c) for the three datasets. All potential combinations of variables for each dataset are presented in Table 3. Models were ranked using the «dredge» function («MuMIn» package; Bartoń, 2022). Following Burnham and Anderson (2002), the model with the lowest AIC_c value and a difference of AIC_c (ΔAIC_c) > 2 when compared with the next best model was considered to be the best. Following Johnson and Omland (2004), model performance was assessed using Akaike weights (w_i). Model assumptions (residual normality, homogeneity, independence) were checked with diagnostic functions («plot» and «qqnorm»). The degree of model fit was reported by using the McFadden's R-Squared metric. Differences between colonies were then identified with Estimated Marginal Means (EMMs; «emmeans» package; Length, 2023) following Bond and Diamond (2009a). Finally, partial residuals were extracted from each best model to obtain predictor effect plots («effects» package; Fox and Weisberg, 2018, 2019). This allowed us to quantify and visualise Hg spatial variation by controlling variation due to both $\delta^{13}C$ and $\delta^{15}N$ values. Year was not included as an explanatory factor for two reasons: (i) year and colony were confounded for most colonies (92%) and (ii) inter-annual differences in Hg concentrations are expected to be negligible over the short term (e.g., several years) at remote locations (Brasso et al., 2014; Carravieri et al., 2016). To confirm this, we quantified inter-annual variation in adult feather Hg concentrations of Adélie penguins at Admiralty Bay (King George/25 de Mayo Island), which were sampled across six consecutive years (between 2005 and 2011). Annual mean feather Hg concentrations ranged from 0.25 to 0.35 $\mu g \cdot g^{-1}$, a low 0.10 $\mu g \cdot g^{-1}$ scope that translated into a marginal statistical significance (one-way ANOVA, $F_{5,114}=2.31$, $p=0.048$).

Visual representation of Hg contamination in the presumed spatial distribution of Adélie penguins

In penguins, body feather Hg concentrations reflect exposure over a large temporal and spatial scale (see previous sections for further details; Brasso et al., 2014; Carravieri et al., 2014a). As a spatial assessment, this study aimed to match visually this year-round Hg accumulation with the presumed, year-round spatial distribution of Adélie penguins. Since the individuals sampled in this study were not tracked precisely with geolocators, their maximum distribution (*i.e.*, including all individual tracks) during the non-breeding season was extracted from published studies (Fig.S1). Tracking data were available for six Antarctic colonies (Ballard et al., 2010; Clarke et al., 2003; Davis et al., 1996; Dunn et al., 2011; Erdmann et al., 2011; Hinke et al., 2015; Takahashi et al., 2018; Thiébot et al., 2019), resulting in a large geographical coverage that can be associated with feather Hg concentrations (Fig. S1). In addition, we used three regionally important habitat characteristics that shape penguins' spatial distribution:

(i) the Antarctic Polar Front (PF). The PF represents an ecologically meaningful limit for the distribution of Adélie penguins (northernmost limit), as they exploit marine resources within the Antarctic Zone, remaining solely south of the PF. Following Freeman and Lovenduski (2016), the PF was defined here as the average of its weekly positions during the 2002−2014 period, with a resolution of 0.25°.

(ii) the Marginal Ice Zone (MIZ). Within the Antarctic Zone, Adélie penguins are closely tied to sea ice, which is their main habitat year-round;they migrate to the MIZ (i.e., the transitional zone between consolidated pack ice and open water) to forage during the non-breeding season (Ballard et al., 2010; Dunn et al., 2011; Takahashi et al., 2018). Therefore, the position of the MIZ can be expected to reflect the birds' presumed distribution during their non-breeding period. Following previous work (Bliss et al., 2019; Meier et al., 2021), the MIZ was defined here as areas included within the maximum annual sea ice extent (*i.e.*, in September) covered by at least 15% of ice , averaged on daily sea ice concentrations over the 2003-2022 period (which encompasses our sampling years; AMSR-E/ASMR2, 3.125 km resolution, downloaded in March 2023 on https://www.seaice.uni-bremen.de; Spreen et al., 2008).

(iii) the 1000 m isobath. To distinguish between neritic (*i.e.*, over the deep Antarctic shelf) and oceanic (*i.e.*, open-ocean) environments that Adélie penguins may exploit, the 1000 m isobath appeared as a key habitat feature (data downloaded in March 2020 from https://www.ncei.noaa.gov/products/etopo-global-relief-model; Bed Rock ETOPO1; 1 arc-min resolution; Amante and Eakins, 2009).

Maps representing these three environmental characteristics with the averaged Hg concentrations for both adults and chicks were computed using Python (Version 3.7.6).

RESULTS

Spatial differences in feather Hg concentrations and isotopic values (unifactorial analyses)

In adult Adélie penguins, individual feather Hg concentrations ranged from 0.06 to 1.35 $\mu g \cdot g^{-1}$ dw (Table 1). Unifactorial analyses revealed that mean values were significantly different between colonies (Kruskal-Wallis, $\chi^2(23)$ =255.7, p<0.0001, n=485). Specifically, seven colonies differed from all others (Pairwise Wilcoxon, p<0.001): five colonies from Terra Nova Bay and Ross Island (*i.e.*, highest averaged concentrations; mean: 0.80, range: 0.46–1.35 $\mu g \cdot g^{-1}$) and two colonies from Joinville Island Group (*i.e.*, lowest averaged concentrations; mean: 0.21, range: 0.06–0.48 $\mu g \cdot g^{-1}$) (Fig.2a).

Individual feather $\delta^{13}C$ values ranged from –27.4 to –19.9 ‰ (Table 1) and mean values differed among colonies (Kruskal-Wallis, $\chi^2(23)$ =226.53, p<0.0001, n=485), with those from the Antarctic Peninsula, Seymour Island and Joinville Island Group (mean: –22.6, range: –25.9, –19.9 ‰) differing from the others (mean: –24.8, range: –27.4, –

22.4 ‰; Fig.2b). Individual feather $\delta^{15}N$ values ranged from 8.0 to 12.8 ‰ (Table 1) and mean values differed among colonies (Kruskal-Wallis, $\chi^2(23) = 249.9$, $p<0.0001$, n=485), with the lowest and highest $\delta^{15}N$ values recorded in Seymour Island (mean: 8.83, range: 8.0–9.2 ‰) and Terra Nova Bay (mean: 10.9, range: 9.0–12.8 ‰), respectively (Fig.2c).

Drivers of feather Hg concentrations (multifactorial analyses)

Results from model selections are presented in Table 3. For all adults (n=485), the best model included $\delta^{13}C$ and $\delta^{15}N$ values and the colony as significant predictors (Table 3), and explained 61% of the observed variation in feather Hg concentrations (Table 4). Feather Hg concentrations decreased and increased with $\delta^{13}C$ ($\beta \pm$ SE: –0.03 ± 0.006; CI: –0.05, –0.02) and $\delta^{15}N$ values ($\beta \pm$ SE: 0.09 ± 0.01, CI: 0.06–0.11), respectively. When accounting for $\delta^{13}C$ and $\delta^{15}N$ values, the colony effect plot showed that: (i) most colonies (71%) exhibited feather Hg concentrations close to the circumpolar average (*i.e.*, 0.45 $\mu g \cdot g^{-1}$; all colonies combined), and (ii) colonies from the Ross Sea had higher Hg concentrations than all other sites (Fig.5). These results are reinforced by the EMMs (Table 5).

For sexed adults (n=231), two models met the selection criteria for the best model (i.e., $\Delta AIC_c<2$), weighing together 90% of all models (Table 3). The first best model ($\Delta AIC_c=0$) included $\delta^{13}C$ and $\delta^{15}N$ values and colony, but did not include sex as a significant predictor (Table 3). The second best model ($\Delta AIC_c=1.2$) included $\delta^{13}C$ and $\delta^{15}N$ values, colony and sex (Table 3). Because it was 1.8 times more powerful than the second one, the first best model was selected as the final best model. This model explained 67% of the measured variation in feather Hg concentrations (Table 4). Feather Hg concentrations strongly decreased and increased with $\delta^{13}C$ ($\beta \pm$ SE: 0.14 ± 0.06; CI: 0.03–0.26; Gamma inverse) and $\delta^{15}N$ values ($\beta \pm$ SE: –0.29 ± 0.06; CI: –0.40, –0.17; Gamma inverse), respectively. Spatial differences are provided with the EMMs in Table S6. Despite no statistical difference between sexes (see Table S5 for more details), differences were observed for Hg concentrations, $\delta^{13}C$ and $\delta^{15}N$ values (Fig.S2). Regarding Hg concentrations, females had lower concentrations than males at six colonies (Queen Maud Land: Hukuro Cove, and the Ross Sea: Adélie Cove, Edmonson Point, Inexpressible Island, Cape Crozier and Cape Royds). In contrast, females had higher concentrations than males at two other colonies (Adélie Land and King George/25 de Mayo Island), respectively. Regarding $\delta^{13}C$ values (Fig.S2), there was no clear difference between sexes in five colonies. Compared to males, females had lower values in one colony (Edmonson Point) and higher values in two colonies from the Ross Sea (Adélie

Cove and Cape Royds). For $\delta^{15}N$ values, females and males had similar values in two colonies (Cape Crozier and Admiralty Bay), but females had lower mean values in five colonies (Hukuro Cove, Adélie Cove, Edmonson Point, Inexpressible Island and Cape Royds) and higher mean values in one colony (Dumont d'Urville) compared to males (Fig.S2).

For the dataset including fledging chicks and adults from the same colonies (n=113), the best model included $\delta^{13}C$ and $\delta^{15}N$ values, colony, age class and their interaction as significant predictors (Table 3), explaining 79 % of the observed variation in feather Hg concentrations (GLM, Table 4). Feather Hg concentrations were strongly and positively driven by $\delta^{15}N$ values (β ± SE: –0.58 ± 0.13; CI: –0.84, –0.33; Gamma inverse). In contrast, $\delta^{13}C$ values were positively but only marginally related to feather Hg concentrations (small effect size, Table 4). In chicks, individual Hg concentrations ranged from 0.07 to 0.63 $\mu g \cdot g^{-1}$ (Table 2), which is 1.8–3.5 times lower than those measured in adults at any colony (Table 1), with the exception of Dumont d'Urville in 2011/2012 (Fig.3a, Table 6). Chick feather $\delta^{13}C$ values ranged from –25.6 to –20.2 ‰, with outlying values in Marambio (Seymour Island) compared to other colonies (Fig.3b). Chick $\delta^{15}N$ values varied from 7.2 to 12.9 ‰ (Table 2), with an average 3‰ difference between East and West Antarctic colonies (Fig.3c).

DISCUSSION

The Southern Ocean hosts some of the most extreme and least accessible environments on Earth. As such, it has commonly been considered to be a pristine ocean, free from substantial anthropogenic contamination. Nevertheless, latitudinal gradients in persistent contaminants, such as Hg, were previously described across the Southern Ocean (Carravieri et al., 2014b; Renedo et al., 2020), posing potential threats for its marine ecosystems. Specifically, lower Hg concentrations were observed in Antarctic compared to subantarctic and subtropical seabirds. Whether this Hg gradient is locally restricted or widespread in the Southern Ocean is still unknown. Here, we studied the Adélie penguin as a circumpolar bioindicator species to reveal ocean-wide patterns in Hg contamination across Antarctic marine ecosystems. To the best of our knowledge, this study is the first to use a single bioindicator and document Hg contamination over such a large spatial scale, encompassing a total of 24 colonies around Antarctica (both continental and maritime). This circumpolar assessment revealed notable variation in feather Hg concentrations of Adélie penguins, with a hotspot in the Ross Sea (Victoria Land). Drivers of this spatial variation involved both the feeding ecology and colony location.

Circumpolar Hg contamination in adult Adélie penguins

Overall, feather Hg concentrations found in this study were similar to those reported by previous studies on Adélie penguins (Table 1). In the Antarctic Zone, Adélie penguins exhibited lower Hg concentrations (0.45 $\mu g \cdot g^{-1}$) compared to chinstrap (*Pygoscelis antarcticus*; 0.71 $\mu g \cdot g^{-1}$), gentoo (*P. papua*; 1.34 $\mu g \cdot g^{-1}$) and emperor (*Aptenodytes forsteri*) penguins (1.37 $\mu g \cdot g^{-1}$) on average (Table S1). This was also the case when comparing with: other penguin species elsewhere in the Southern Ocean (Table S1), such as the southern rockhopper (*Eudyptes chrysocome*; 2.03 $\mu g \cdot g^{-1}$), king (*A. patagonicus*; 2.19 $\mu g \cdot g^{-1}$) and macaroni (*E. chrysolophus*; 2.73 $\mu g \cdot g^{-1}$) penguins; and with other flying seabirds from the Southern Ocean, including storm petrels (5.47 $\mu g \cdot g^{-1}$; Pacyna et al., 2019) or albatrosses (22.14 $\mu g \cdot g^{-1}$; Bustamante et al., 2016). Our results thus confirm that the overall Hg concentrations are consistent around the Antarctic continent. We propose that the Adélie penguin, which is also a relevant indicator species for CCAMLR, is a good indicator for Hg contamination in Antarctic marine food webs.

In adult Adélie penguins, body feather Hg concentrations reflect a large temporal and spatial scale of exposure (see Material and Methods for further details; Brasso et al., 2014; Carravieri et al., 2014a). During the breeding season, Adélie penguins are central place foragers (Ainley, 2002): they forage in a restricted area immediately adjacent to the colony, within a few hundred kilometres (Ainley et al., 2004; Davis and Miller, 1992; Michelot et al., 2021; Riaz et al., 2021). During the non-breeding period, they travel several hundred to thousand of kilometres away from their breeding grounds (e.g., 500–10,000 km on average; Ballard et al., 2010; Clarke et al., 2003; Davis et al., 1996; Dunn et al., 2011; Erdmann et al., 2011; Hinke et al., 2015; Takahashi et al., 2018; Thiébot et al., 2019).and are assumed to forage in the MIZ (Dunn et al., 2011; Wilson et al., 2001). In the absence of precise tracking positions, feather Hg concentrations could be qualitatively associated with a large spatial region, encompassing most of the Antarctic Zone from the Antarctic Peninsula to the Ross Sea, thanks to published tracking studies (Fig.S1). At the circumpolar scale, our results revealed spatial variation in feather Hg concentrations, which were the lowest in West Antarctica (*i.e.*, maritime Antarctica: King George/25 de Mayo, Seymour and Joinville Islands, and the Antarctic Peninsula), intermediate in East Antarctica, and the highest in the Ross Sea (Fig.2a and Fig.4).

As expected, penguin feeding ecology was a major driver of among colony variation, as indicated by the results of model selection (Table 3). Trophic position was a significant predictor of feather Hg concentrations, as showed by the higher $\delta^{15}N$ values in the Ross Sea (Fig.2c). This suggests that penguins foraged at higher trophic position at these locations. Adélie penguins are strongly associated with sea-ice environments, both during the breeding (Emmerson and Southwell, 2008; Guen et al., 2018; Kokubun et al., 2021) and non-breeding seasons (Emmerson and Southwell, 2011). They feed preferentially in waters covered by 10 to 80% sea ice, but also in the open sea (Ballard et al., 2019; Cottin et al., 2012; Guen et al., 2018; Michelot et al., 2020). In these habitats, their diet comprises different euphausiid crustaceans, mainly the Antarctic krill (*Euphausia superba*) and smaller amounts of the ice krill (*Euphausia crystallorophias*) (Tierney et al., 2009). Their diet also includes fish species such as the Antarctic silverfish (*Pleuragramma antarcticum)* in different proportions according to colony location and season (Ainley et al., 1998; Ainley, 2002). Thus, their diet may vary significantly according to the geographical localization of the colony, but also the year (Tierney et al., 2009). For example, Adélie penguins feed almost exclusively on Antarctic krill along the Antarctic Peninsula and in the Scotia Sea (Coria et al., 1995; Juáres et al., 2018; Lynnes et al., 2004). In East Antarctica, they have a mixed diet depending on feeding habitat: fish and ice krill in neritic waters (continental shelf) *versus* Antarctic krill in pelagic waters (shelf break; Green and Johnstone, 1988; Kent et al., 1998; Puddicombe and Johnstone, 1988; Watanuki et al., 1997; Wienecke et al., 2000). In contrast, Adélie penguins from the Ross Sea consume higher proportions of Antarctic silverfish (Ainley et al., 1998; Olmastroni et al., 2020), which are abundant in continental shelf waters (Gon and Heemstra, 1990). Yet, the silverfish is also a zooplankton predator itself and thus exhibits a higher trophic position than krill (Everson, 2000; Hodum and Hobson, 2000; Polito et al., 2011). Hence, a higher consumption of silverfish could explain the higher Hg concentrations observed in Adélie penguins from Victoria Land (Ross Sea). This is supported by previous studies showing that Hg concentrations in several species of Antarctic fish, including the Antarctic silverfish, were 4 to 20 times higher than in other krill species (Polito et al., 2016; Seco et al., 2021, 2019; Sontag et al., 2019).

Nevertheless, the diet of adult Adélie penguins (as detailed above) has been studied mainly determined during the chick-rearing season, when individuals are directly accessible on land, but scarcely during the non-breeding season (also the period of Hg exposure). Given the lack of dietary information available for the non-breeding period, we assumed here that Adélie penguins use similar marine resources during both periods (Polito et al., 2016; Tierney et al., 2009). However, seasonal variation in feeding ecology of Adélie penguins cannot be excluded and could thus influence feather Hg concentrations.

Five penguins from Hukuro Cove and Brown Bluffs were excluded from analyses (Table S4) because they had unexpected high positive $\delta^{13}C$ values for Antarctic environments (Carravieri et al., 2014b; Cherel and Hobson, 2007) and high $\delta^{15}N$ values for this species. Since it is unlikely that these individuals were associated with northern ecosystems, such values would rather indicate that the birds had foraged in more coastal/benthic habitats (Cherel et al., 2011), possibly resulting in high feather Hg concentrations. One way to better understand why these five individuals had different isotopic signatures would be to perform Compound-Specific Stable Isotope Analysis of Amino-Acids (CSIA-AA). Indeed, CSIA-AA enables to distinguish between source (*i.e.,* baseline) and trophic $\delta^{15}N$ values. In other words, it could clarify whether the $\delta^{15}N$ baseline is different (environmental driver) or whether the diet is the major driver (trophic driver). Similarly, CSIA-AA analyses are likely to help clarify whether the differences in $\delta^{15}N$ values observed in the Ross Sea came from different $\delta^{15}N$ baseline, trophic position or both, and how this could translate into a higher Hg contamination.

Regional colony location was also associated with feather Hg concentrations (Table 3), suggesting that environmental factors are also involved in explaining spatial differences in feather Hg concentrations. When accounting for feeding ecology (*i.e.,* $\delta^{13}C$ and $\delta^{15}N$ values), colonies from Victoria Land (Ross Sea) still appeared to have higher Hg concentrations than all other colonies (Fig.5). Hence, penguins in the Ross Sea have a disproportionately higher Hg concentrations than would be expected on the basis of their diet alone. Two non-exclusive factors could explain this result: (1) volcanism and (2) katabatic winds. The West-Antarctic Rift System runs from the base of the Antarctic Peninsula through the Weddell Sea to the Ross Sea (Rocchi et al., 2003), resulting in numerous volcanoes present in Western versus Eastern Antarctica. Despite low activity levels, two main active volcanoes border the coast of the Ross Sea: Mount Erebus and Mount Melbourne, which are located on Ross Island and in Terra Nova Bay, respectively (Behrendt, 1990; Edwards and Smellie, 2016; Ferraccioli et al., 2000; Global Volcanism Program, 2022). Since volcanoes represent a primary source of Hg to the atmosphere (Grasby et al., 2019), they are likely to constitute a local source of Hg for the ocean and associated marine food webs as well. On the other hand, katabatic winds may also influence Hg deposition in the Ross Sea. Katabatic winds are strong winds that blow from the large and elevated Antarctic ice sheets toward the coast and represent a major environmental feature in Antarctica (Parish, 1988; Parish and Cassano, 2003) that can transport dust and debris. The Ross Sea is strongly exposed to katabatic winds (Turner, 2015) and in Terra Nova Bay, regions exposed to strong katabatic winds showed enhanced Hg deposition on the coast (Bargagli, 2008, 2016). Consequently, by carrying air masses originating from the Antarctic continent towards coastal regions, katabatic winds could represent a local natural source of Hg in marine ecosystems of the Ross Sea. Still, the biochemical cycle of Hg is complex and includes several chemical processes and biological transformations with both abiotic and biotic interactions (Chételat et al., 2022; McKinney et al., 2022): methylation/demethylation, redox reactions, MeHg production, bioavailability and transfer through marine food webs, especially in polar environments with the influence of sea ice (Cossa et al., 2011). The presence of sea ice, which harbour microbial sources of MeHg in the Southern Ocean (Gionfriddo et al., 2016; Yue et al., 2023), may thus influence Hg contamination in Antarctic marine food webs. Further research is needed to disentangle the biochemical processes at the circumpolar scale, but also those that could result in higher year-round Hg exposure in Adélie penguins from the Ross Sea.

Because sample collection is challenging in Antarctic environments, feathers in this study were collected across a relatively large time period (between 2005 and 2021). Even if interannual variations were very low in one colony where adult Hg concentrations were available for six consecutive years (*i.e.*, Admiralty Bay, King George/25 de Mayo Island), observed spatial variations could result from the combination of both spatial and temporal variations. It is worth noting that Hg concentrations in two colonies were different from those reported previously. In Queen Maud Land, feather Hg concentrations in the 2010s were five times higher than in the 1980s and the 1990s (Honda et al., 1986; Yamamoto et al., 1996; Table 1). In Adélie Land, feather Hg concentrations dropped by 30% between 2006 and the following years (2011 and 2017; Carravieri et al., 2016; this study; Table 1). However, this temporal difference (0.2 $\mu g \cdot g^{-1}$) is still low compared to the spatial difference measured among adult Hg concentrations (1.2 $\mu g \cdot g^{-1}$). Further studies should thus investigate mid- and long-term Hg trends in Antarctic food webs, for instance by increasing the temporal resolution in Hg monitoring.

Potential sex-specific Hg concentrations

In seabirds, sexual segregation in diet has been suggested as a strategy to reduce intra-specific competition (Bearhop et al., 2006; Forero et al., 2002; González-Solís et al., 2000; Phillips et al., 2011). In Adélie penguins, diet segregation between sexes was observed during the breeding season: females foraged for more krill than fish in more offshore, pelagic waters, in contrast to males which fed equally on both prey types in more inshore, benthic waters (Clarke et al., 1998; Colominas-Ciuró et al., 2018). In theory, such sexual segregation in diet should therefore be reflected in stable isotopes and then in Hg concentrations. In our study, no clear sexual segregation could be deduced from the observed feather $\delta^{13}C$ and $\delta^{15}N$ values (Fig.S2b and c). However, differences between sexes in feather Hg concentrations could be noticed when differences in either $\delta^{13}C$ or $\delta^{15}N$ values or both were present. Feather Hg concentrations were similar between sexes at Adélie Cove (Terra Nova Bay) and Admiralty Bay (King George/25 de Mayo Island), as previously reported (Polito et al., 2016). In contrast, females had lower Hg concentrations in Queen Maud Land (Hukuro Cove) and the Ross Sea, including Edmonson Point and Inexpressible Island (Terra Nova Bay), and Cape Crozier and Cape Royds (Ross Island). These results were similar to previous studies on Adélie penguins from the Ross Sea (Pilcher et al., 2020), but also in other penguin species (*i.e.*, Gentoo and Macaroni penguins) from South Georgia/Islas Georgias del Sur (Becker et al., 2002). Although moult is the major process responsible for Hg elimination in males, egg production represents an additional route (Bond and Diamond, 2009b; Braune and Gaskin, 1987), that could lead to lower Hg concentrations in females.

Despite these potential differences between sexes, sex did not significantly drive spatial variation in adult Hg contamination, according to the first best model (Table 3). Indeed, this model included $\delta^{13}C$ and $\delta^{15}N$ values and colony location. However, a second model met the selection criteria for the best model (although its weight was lower), which included sex as a predictor in addition to $\delta^{13}C$ and $\delta^{15}N$ values and colony location (Table 3). Unfortunately, information on penguin sex was not available for all individuals. Models were thus run on a dataset that only included half of the total sample size. Complete sexing analyses, either determined through morphological or molecular approaches, should help to further investigate differences in Hg contamination between sexes and its drivers (either extrinsic or intrinsic), on a circumpolar scale. Whether sexual segregation in diet and Hg contamination are related during the breeding and the non-breeding season warrants further research.

Comparing Hg contamination on different spatio-temporal scales with adult and chick feathers

Chick and adult feathers reflect different temporal and spatial scales: local accumulation during a few months during the breeding season *versus* year-round accumulation including their entire distribution (breeding and non-breeding), respectively. In general, chicks exhibit lower feather Hg concentrations relative to adults, as a result of their shorter exposure period (Carravieri et al., 2014c). Our results were coherent with this explanation, with feather concentrations in chicks being 1.8 to 3.6 times lower than in adults on average (Fig.3).

Feather Hg concentrations were driven by the age class (adult or fledging chick), the trophic ecology (indicated by $\delta^{13}C$ and $\delta^{15}N$ values) and colony location (Table 3). In most colonies, chicks exhibited equivalent or higher feather $\delta^{15}N$ values than adults, suggesting that they were fed with similar prey or with prey of higher trophic position across colonies (for example with higher proportions of fish compared to krill; Cherel, 2008). There was one exception to this pattern: in Dumont d'Urville (Adélie Land; in 2011), average feather Hg concentrations were similar between adults and chicks (Table 6), translating into a similar contamination status between age classes. In 2011, sea ice was quite high around the colony (60%) during the chick-rearing period and quite homogenous at a larger scale (Michelot et al., 2021). Whether these sea-ice conditions could be associated with higher Hg exposure for both adults and chicks would deserve further research.

Importantly, Terra Nova Bay (and hence the Ross Sea) exhibited the highest feather Hg concentrations in both chicks and adults (Fig.4), which reinforces the higher exposure year-round in this region compared to other areas. Since foraging trips are shorter during the chick-rearing period, this also suggests that Hg contamination may be local.

Conclusions and perspectives:

This study provides a unique assessment of Hg contamination in Antarctic marine food webs, by using the Adélie penguin as a circum-Antarctic bioindicator. Feathers represent a valuable, non-destructive and non-invasive monitoring tool, in complete agreement with Antarctic Treaty protocols, to examine the variation in Hg contamination across temporal and spatial scales. Feather Hg concentrations detected in Adélie penguins ($< 2\ \mu g \cdot g^{-1}$) were below toxicity thresholds recognized for seabird feathers ($1.6–10\ \mu g \cdot g^{-1}$; Ackerman et al., 2016; Chastel et al., 2022). Currently, this suggests low risks of toxicity for this species, although toxic effects may arise at low Hg concentrations, particularly in combination with other stressors (*e.g*., other contaminants, environmental changes, diseases; Grunst et al., 2023; Provencher et al., 2016). At the circumpolar scale, Hg contamination was relatively homogeneous across regions. This is consistent with the circumpolar structure of the Southern Ocean, which is characterized by a unique stratification of annular fronts and water masses encircling the Antarctic Continent (Carter et al., 2008). This reinforces the suitability of Adélie penguins as bioindicator species for Antarctic marine ecosystems.

Trophic ecology (indicated by feeding habitat and trophic position) was crucial to explain spatial variations of Hg contamination. The Hg hotspot observed in the Ross Sea was associated with higher trophic position of Adélie penguins, probably due to a higher proportion of fish in their diet. This reinforces the need to account systematically for the diet when monitoring Hg contamination in seabirds, especially at such large spatial scales.

Thanks to published tracking studies, feather Hg concentrations could be qualitatively associated with a large spatial region, encompassing most of the Antarctic Zone, from the Antarctic Peninsula to the Ross Sea. However, detailed spatial information of penguin movements during their annual cycle is essential to quantify precisely the spatial variation in their Hg exposure. The Arctic Monitoring and Assessment Program (AMAP) provides a circumpolar and long-term assessment of Hg contamination in marine ecosystems for the entire Arctic Ocean. In this program, Hg contamination was associated to the spatial and seasonal distributions of seabirds thanks to biologging. In a similar way, such work in the Southern Ocean would be substantially improved by the deployment of individual tracking devices, allowing connection to be made between spatial and seasonal distributions of penguins, at the individual level, with contaminants at the ocean scale.

Through Adélie penguins, this work documented circumpolar Hg contamination in the epipelagic compartment of the Southern Ocean. A complementary approach could investigate the mesopelagic compartment, where high MeHg concentrations were recorded (Cossa et al., 2011), by studying the circumpolar breeding emperor penguin (*Aptenodytes forsteri*), which mainly feeds on prey of higher trophic position in both epipelagic and mesopelagic waters (Cherel, 2008; Wienecke and Robertson, 1997). Such large-scale monitoring is fundamental for international monitoring programs, such as the Global Mercury Assessment from the United Nations, to assess the effectiveness of the Minamata Convention on Mercury. Renewing a large-scale assessment such as that presented here on a regular basis (every few years or decade for example) is highly recommended to monitor the contamination status of Antarctic marine food webs over time and to further investigate global trends, especially in the context of climate change.

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval

In this work, feather sampling relied on several existing monitoring programs and were carried out at all sites under appropriate permits. More specifically, (i) animal handling and sample collection for Australian stations (Mawson, Davis and Casey stations) were approved by the Australian Antarctic Division animal ethics committee and ATEP environmental approvals. (ii) Fieldwork in Adélie Land was approved by the Conseil des Programmes Scientifiques et Technologies Polaires of the Institut Polaire Français Paul Emile Victor (IPEV), and procedures were approved by the Animal Ethics Committee of IPEV. (iii) Fieldwork in Terra Nova Bay was approved under permission released from the Programma Nazionale di Ricerche in Antartide (PNRA). All sampling followed SCAR's Code of Conduct for the Use of Animals for Scientific Purposes in Antarctica (https://www.scar.org/policy/scar-codes-of conduct). (iv) The Instituto Antártico Argentino – Dirección Nacional del Antártico (PINST-05) provided financial and logistical support to carry out the Argentine Antarctic campaign. The field work was carried out under the permit granted by the Dirreción Nacional del Antártico (Environmental Management Office). (v) Animal handling and sample collection at Signy Island was approved by the British Antarctic Survey Animal Welfare and Ethical Review Board, and permission was granted by the British Foreign and Commonwealth Office on behalf of HM Secretary of State, under Sections 12 and 13 of the Antarctic Act, 1994, 2013.

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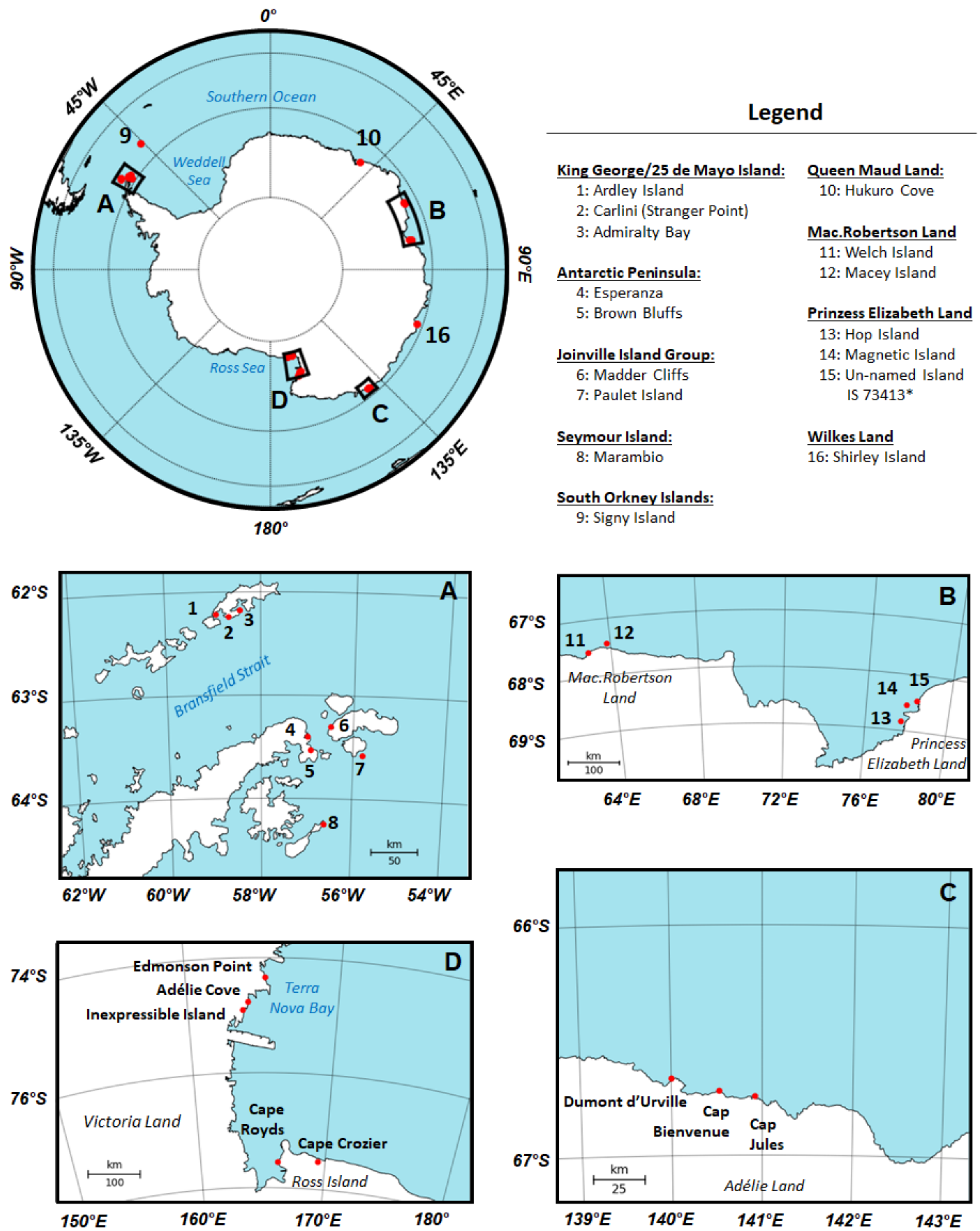

Fig.1 Sampling colonies of the present study (n=24), where feathers of Adélie penguins (*Pygoscelis adeliae;* both adult and chick) were collected during the breeding season, between 2005 and 2021 (see **Tables 1** and **2** for further details)

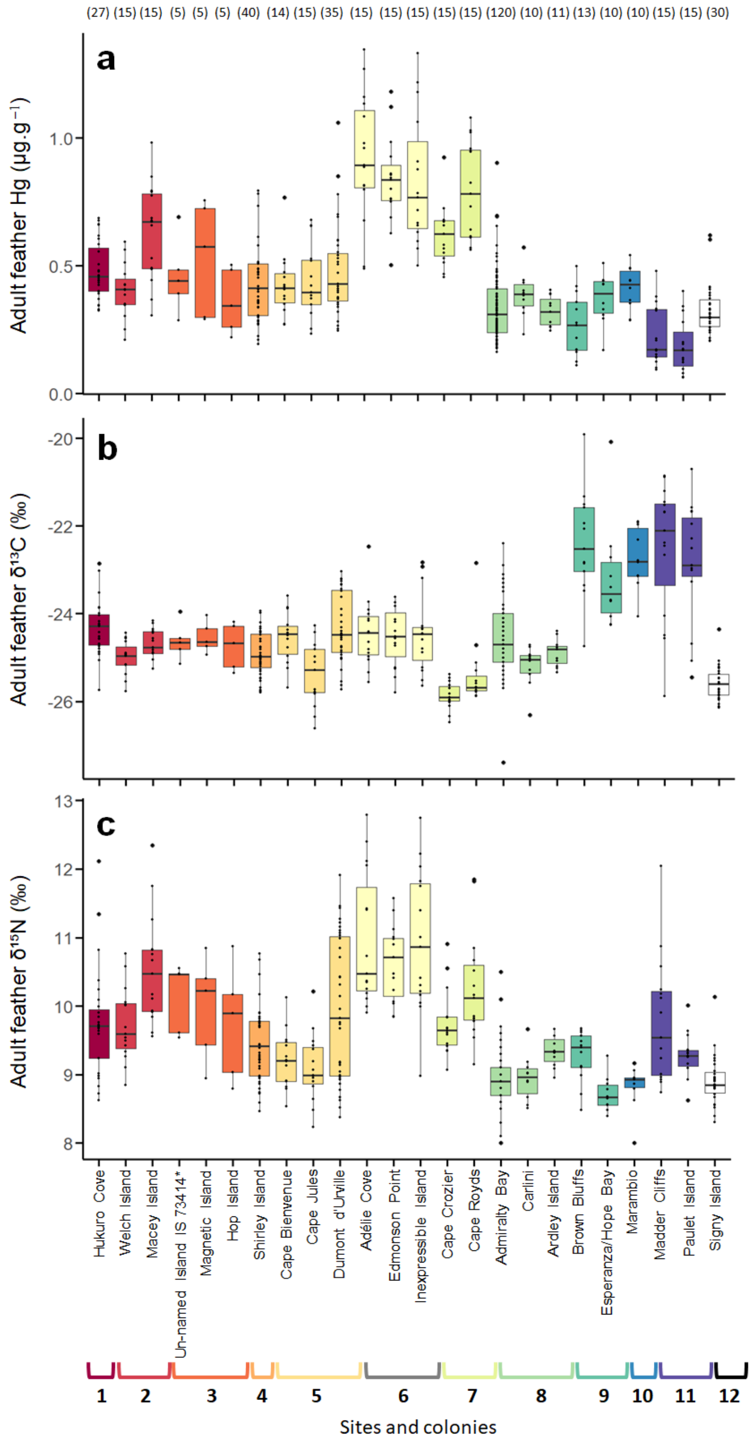

Fig.2 Spatial comparison of feather (**a**) Hg concentrations, (**b**) $\delta^{13}C$ and (**c**) $\delta^{15}N$ values in adult Adélie penguins (*Pygoscelis adeliae*) collected in 24 Antarctic colonies, represented by a clockwise colour gradient (from East-Antarctica in dark red to South Orkney Islands in grey). Feather $\delta^{13}C$ and $\delta^{15}N$ values are proxies for penguin feeding habitat and trophic position, respectively. Numbers in brackets (top) represent sample sizes for each colony. Numbers (bottom) refer to the sites where the sampling colonies are located: (1) Queen Maud Land, (2) Mac.Robertson Land, (3) Princess Elizabeth Land, (4) Wilkes Land, (5) Adélie Land, Victoria Land: (6) Terra Nova Bay, (7) Ross Island; (8) King George/25 de Mayo Island, (9) Antarctic Peninsula, (10) Seymour Island, (11) Joinville Island Group and (12) South Orkney Islands. Individual values (smaller dots) are presented with boxplots, representing median values (midlines), errors bars (whiskers) and outliers (black dots outside whiskers).

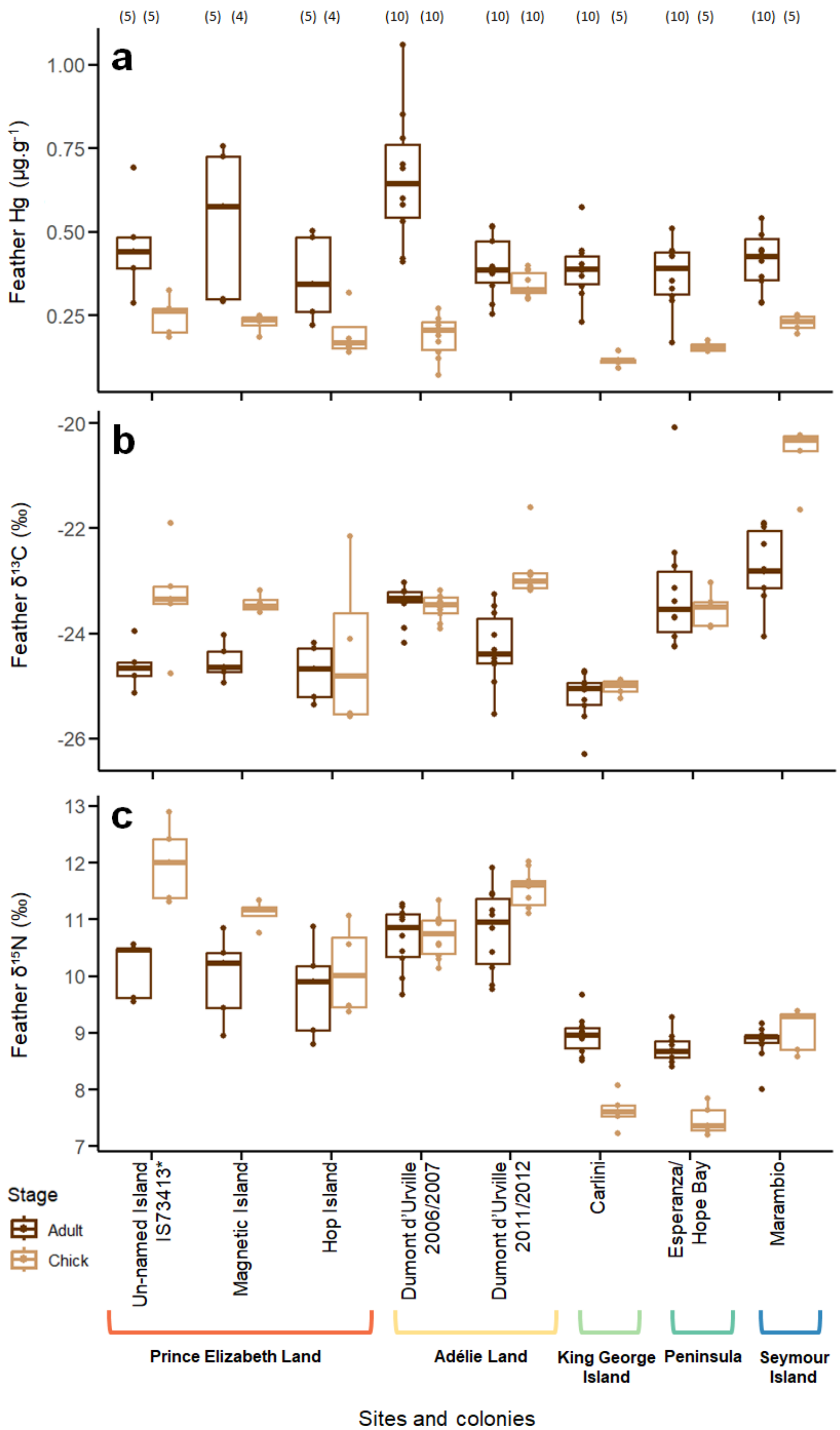

Fig. 3 Adult and chick comparison in feather **(a)** Hg concentrations, and **(b)** $\delta^{13}C$ and **(c)** $\delta^{15}N$ values in Adélie penguins (*Pygoscelis adeliae*) from eight Antarctic colonies. Adult and chicks are represented in dark and light brown, respectively. Feather $\delta^{13}C$ and $\delta^{15}N$ values are proxies for penguin feeding habitat and trophic position, respectively. Individual values (smaller dots) are presented with boxplots, representing median values (midlines), errors bars (whiskers) and outliers (black dots outside whiskers). Numbers in brackets indicate sample sizes for each age class and colony

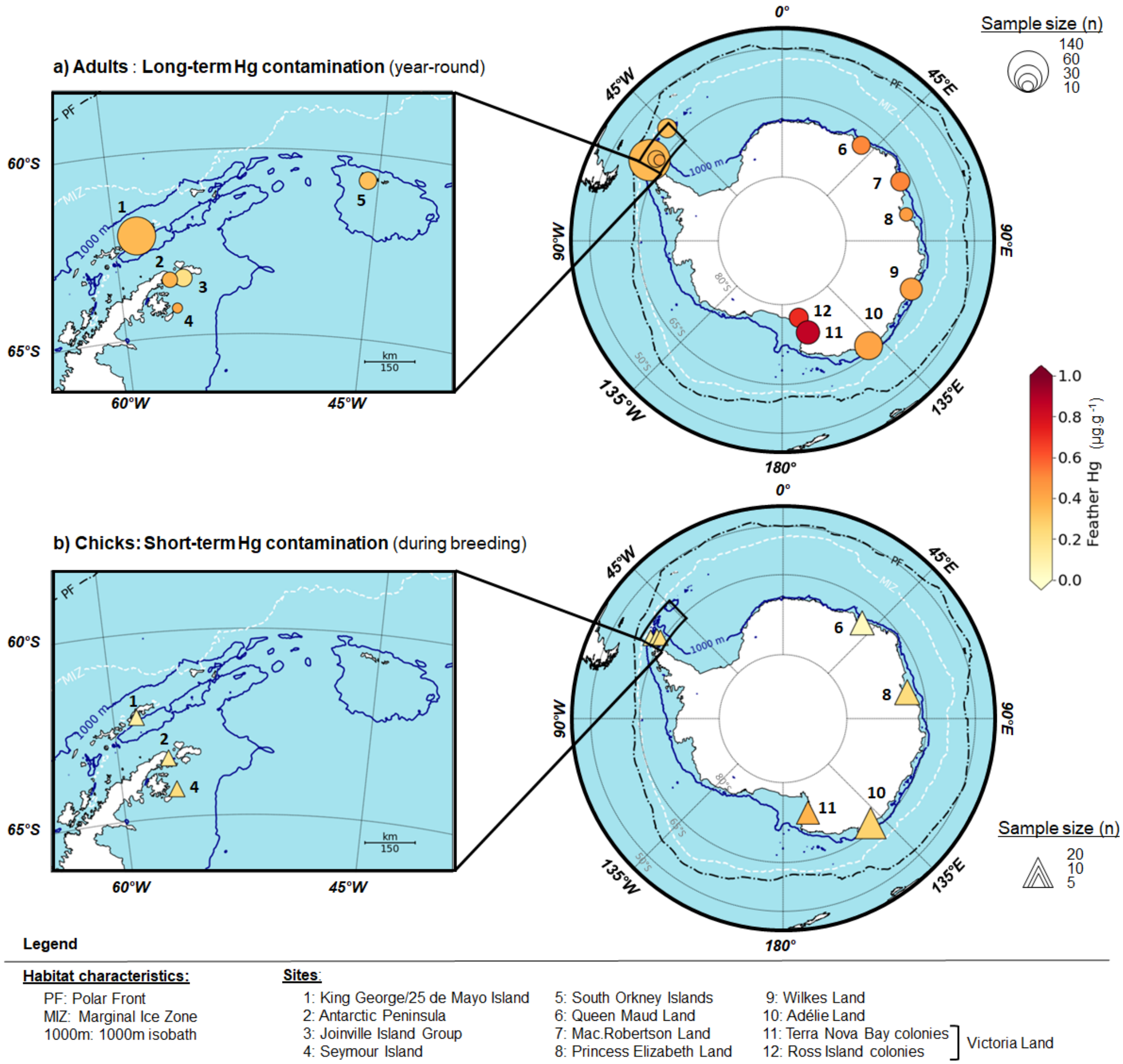

Fig.4 Spatial variations in feather Hg concentrations of (**a**) adult and (**b**) chick Adélie penguins (*Pygoscelis adeliae*) across 24 Antarctic colonies. The colour gradient represents increasing Hg concentrations. Sample sizes (n) are indicated by the size of circles and triangles. The averaged position of the Polar Front (PF, dashed black line) reflects the northernmost limit of Adélie penguin distribution, whereas the Marginal Ice Zone (MIZ, dashed white line) reflects the presumed northern limit of their non-breeding distribution (*i.e.*, maximum sea ice cover extent in September; see **Material and Methods** for further details). The darkblue line indicates the 1000 m isobath

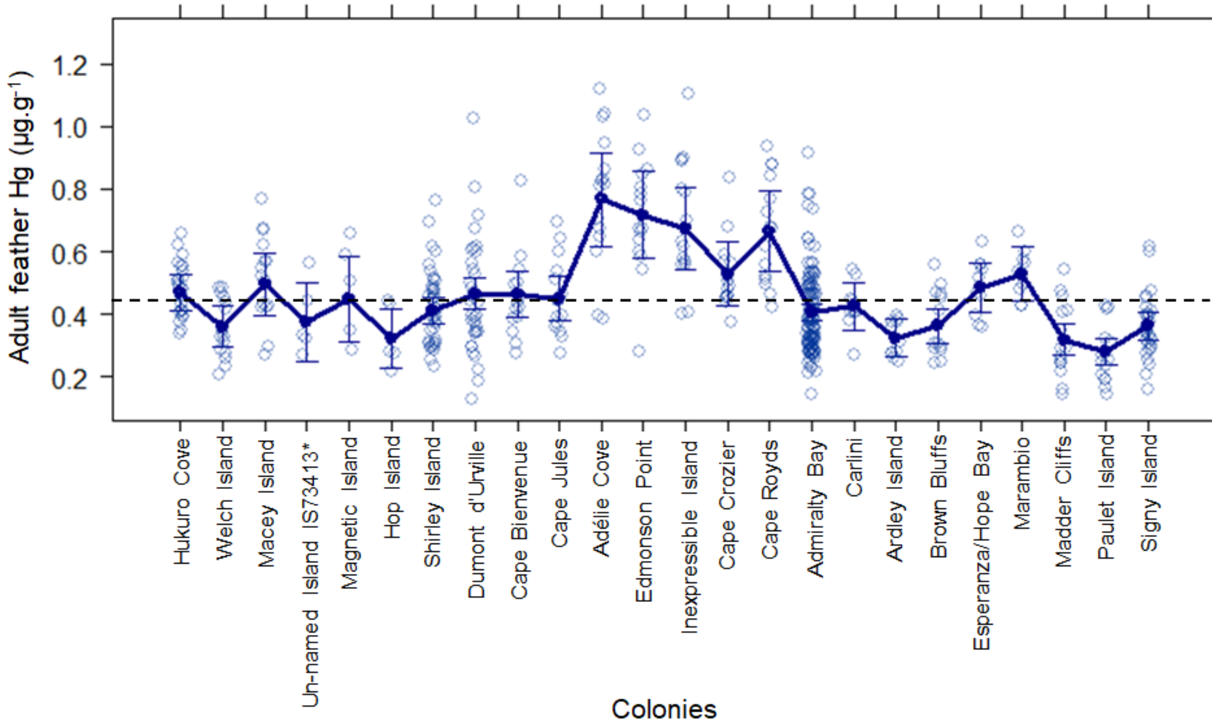

Fig.5 Spatial comparison in feather Hg concentrations in adult Adélie penguins (*Pygoscelis adeliae*) from 24 Antarctic colonies (n=485) when controlled by their feeding ecology (feather $\delta^{13}C$ and $\delta^{15}N$ values). Relationships result from the extraction of partial residuals of the best Generalized Linear Model (GLM; see **Table 3** and **Material and Methods** for further details). Individual data are represented in light blue (open circle). Points (filled circle) are means ± SD. The dark blue line links all mean Hg concentrations. The dashed black line indicates the circumpolar average Hg concentrations (*i.e.*, 0.45 $\mu g \cdot g^{-1}$, all pooled data from all colonies)

TABLES

Table 1. Feather Hg concentrations and stable isotope values from adult Adélie penguins (*Pygoscelis adeliae*) sampled around Antarctica, from both this study (included in the analyses) and literature review (for comparison; not included in the analyses). n indicates sample sizes. Feather $\delta^{13}C$ and $\delta^{15}N$ values are proxies of penguin feeding habitat and trophic position, respectively. Values are expressed as means ± SD with ranges in parentheses.

Sites, colonies	**Season**	**n**	**Feather Hg (µg·g^{-1} dw)**	**Feather $\delta^{13}C$ (‰)**	**Feather $\delta^{15}N$ (‰)**	**References**	**Included**
Continental Antarctica							
Queen Maud Land							
Hukuro Cove	2010/2011	27	0.51 ± 0.15 (0.32–0.92)	–23.9 ± 1.5 (–25.7, –22.9)	10.1 ± 1.4 (8.6–12.1)	This study	Yes
	1990/1991	10	0.09 ± 0.05	–	–	Yamamoto et al. (1996)	No
Rumpa Island	1981/1982	10	0.17 ± 0.05 (0.11–0.27)	–	–	Honda et al. (1986)	No
Mac.Robertson Land							
Welch Island	2019/2020	15	0.40 ± 0.11 (0.21–0.59)	–25.0 ± 0.4 (–25.8, –24.4)	9.7 ± 0.5 (8.8–10.8)	This study	Yes
Macey Island	2019/2020	15	0.63 ± 0.19 (0.31–0.98)	–25.0 ± 0.3 (–25.3, –24.2)	10.5 ± 0.8 (9.6–12.3)	This study	Yes
Princess Elizabeth Land							
Hop Island	2019/2020	5	0.36 ± 0.13 (0.22–0.50)	–24.7 ± 0.5 (–25.4, –24.2)	9.8 ± 0.9 (8.8–10.9)	This study	Yes
Magnetic Island	2019/2020	5	0.53 ± 0.22 (0.29–0.76)	–24.5 ± 0.4 (–24.9, –24.0)	10.0 ± 0.8 (8.9–10.8)	This study	Yes
Un-named Island IS 73413*	2019/2020	5	0.46 ± 0.15 (0.29–0.69)	–24.6 ± 0.4 (–25.1, –24.0)	10.1 ± 0.5 (9.5–10.6)	This study	Yes
Wilkes Land							
Shirley Island	2020/2021	40	0.43 ± 0.15 (0.19–0.79)	–24.9 ± 0.5 (–25.8, –23.9)	9.5 ± 0.6 (8.5–10.8)	This study	Yes
Adélie Land							
Dumont d'Urville	2006/2007	10	0.66 ± 0.20 (0.41–1.06)	–23.4 ± 0.4 (–24.2, –23.0)	10.7 ± 0.6 (9.7–11.3)	Carravieri et al. (2016)	Yes
	2011/2012	10	0.40 ± 0.09 (0.25–0.52)	–24.3 ± 0.7 (–25.6, –23.3)	10.8 ± 0.7 (9.8–11.9)	Carravieri et al. (2016)	Yes
	2017/2018	15	0.41 ± 0.11 (0.25–0.60)	–24.9 ± 0.4 (–25.7, –24.4)	8.9 ± 0.3 (8.4–9.7)	This study	Yes
Cape Bienvenue	2017/2018	14	0.42 ± 0.13 (0.27–0.77)	–24.5 ± 0.6 (–25.7, –23.6)	9.2 ± 0.4 (8.5–10.1)	This study	Yes
Cape Jules	2017/2018	15	0.44 ± 0.14 (0.23–0.68)	–25.3 ± 0.7 (–26.6, –24.3)	9.1 ± 0.5 (8.2–10.2)	This study	Yes
Victoria Land (Ross Sea)							

Terra Nova Bay							
Adélie Cove	2017/2018	15	0.92 ± 0.25 (0.49–1.35)	–24.4 ± 0.8 (–25.6, –22.5)	11.0 ± 1.0 (9.9–12.8)	This study	Yes
Inexpressible Island	2017/2018	15	0.83 ± 0.26 (0.50–1.33)	–24.5 ± 0.9 (–25.6, –22.8)	10.9 ± 1.0 (9.0–12.8)	This study	Yes
Edmonson Point	2017/2018	15	0.83 ± 0.18 (0.50–1.18)	–24.5 ± 0.7 (–25.8, –23.6)	10.6 ± 0.6 (9.8–11.6)	This study	Yes
Ross Island							
Cape Crozier	2019/2020	15	0.62 ± 0.12 (0.46–0.92)	–25.9 ± 0.3 (–26.5, –25.4)	9.8 ± 0.5 (9.1–10.9)	This study	Yes
Cape Royds	2019/2020	15	0.80 ± 0.19 (0.56–1.08)	–25.4 ± 0.8 (–25.9, –22.8)	10.3 ± 0.8 (9.2–11.9)	This study	Yes
Cape Bird	2004–2016	154	0.59 ± 0.17	–	–	Pilcher et al. (2020)	No
Cape Hallett, Cape Adare	2004–2016	20	0.50 ± 0.10	–	–	Pilcher et al. (2020)	No
Multiple sites							
Cape Bird, Cape Hallett, Cape Adare	2004–2016	174	0.59 ± 0.02	–25.3 ± 0.6	9.0 ± 0.7	Pilcher et al. (2020)	No
Maritime Antarctica							
King George/25 de Mayo Island							
Ardley Island	2021/2022	11	0.32 ± 0.06 (0.24–0.41)	–24.9 ± 0.3 (–25.3, –24.4)	9.3 ± 0.2 (9.0–9.7)	This study	Yes
Admiralty Bay	2005/2006	20	0.35 ± 0.16 (0.20–0.90)	–24.9 ± 0.7 (–25.7, –23.3)	8.8 ± 0.4 (8.1–9.7)	Brasso et al. (2013, 2014, 2015)	Yes
	2006/2007	20	0.25 ± 0.12 (0.16–0.70)	–25.0 ± 0.3 (–25.7, –24.6)	8.7 ± 0.3 (8.1–9.6)	Brasso et al. (2013, 2014, 2015)	Yes
	2007/2008	20	0.38 ± 0.11 (0.21–0.65)	–24.8 ± 0.5 (–25.3, –23.9)	8.9 ± 0.3 (8.5–9.7)	Brasso et al. (2013, 2014, 2015)	Yes
	2008/2009	16	0.35 ± 0.08 (0.18–0.47)	–24.6 ± 0.5 (–25.3, –23.8)	8.9 ± 0.5 (8.3–10.5)	Brasso et al. (2013, 2014, 2015)	Yes
	2009/2010	22	0.35 ± 0.14 (0.18–0.69)	–23.9 ± 0.8 (–25.4, –22.4)	9.1 ± 0.4 (8.0–10.1)	Brasso et al. (2013, 2014, 2015)	Yes
	2010/2011	22	0.35 ± 0.14 (0.18–0.69)	–24.1 ± 1.1 (–27.4, –22.4)	9.1 ± 0.4 (8.0–10.1)	Brasso et al. (2013, 2014, 2015), Polito et al. (2016)	Yes
	2005–2011	120	0.34 ± 0.13 (0.16–0.90)	–24.5 0.8 (–27.4, –22.4)	8.9 ± 0.4 (8.0–10.5)	Brasso et al. (2013, 2014, 2015)	Yes
Carlini (Stranger Point)	2019/2020	10	0.39 ± 0.09 (0.23–0.57)	–25.2 ± 0.5 (–26.3, –24.7)	9.0 ± 0.3 (8.5–9.7)	This study	Yes
Hennequin Point, Hannah Point	2013/2014	31	0.40 ± 0.13 (0.16–0.69)	–	–	Souza et al. (2020)	No
Antarctic Peninsula							
Esperanza/Hope Bay	2019/2020	10	0.37 ± 0.10 (0.17–0.51)	–23.2 ± 1.3 (–24.3, –20.1)	8.7 ± 0.3 (8.4–9.3)	This study	Yes

Brown Bluffs	2019/2020	13	0.37 ± 0.30 (0.11–1.22)	–21.8 ± 1.8 (–24.7, –19.9)	9.7 ± 1.1 (8.5–9.7)	This study	Yes
Seymour Island							
Marambio	2019/2020	10	0.41 ± 0.09 (0.29–0.54)	–22.7 ± 0.7 (–24.1, –21.9)	8.8 ± 0.3 (8.0–9.2)	This study	Yes
Joinville Island Group							
Madder Cliffs	2019/2020	15	0.23 ± 0.12 (0.09–0.48)	–22.5 ± 1.5 (–25.9, –20.9)	9.8 ± 0.9 (8.7–12.0)	This study	Yes
Paulet Island	2019/2020	15	0.18 ± 0.11 (0.06–0.40)	–22.9 ± 1.4 (–25.5, –20.7)	9.3 ± 0.3 (8.6–10.0)	This study	Yes
Signy Island							
Gourlay	2021/2022	30	0.33 ± 0.10 (0.21–0.62)	–25.6 ± 0.4 (–26.1, –24.4)	8.9 ± 0.3 (8.3–10.1)	This study	Yes

– No available data (stable isotopes).

*Alpha-numeric identifier in Southwell et al. (2021)

Table 2. Feather Hg concentrations and stable isotope values from Adélie penguin chicks sampled around Antarctica, from both this study (included in the analyses) and a literature review (for comparison; not included in the analyses). n indicates sample sizes. Feather $\delta^{13}C$ and $\delta^{15}N$ values are proxies of penguin feeding habitat and trophic position, respectively. Values are means ± SD with ranges in parentheses.

Sites, colonies	Season	n	Feather Hg ($\mu g \cdot g^{-1}$ dw)	Feather $\delta^{13}C$ (‰)	Feather $\delta^{15}N$ (‰)	References	Included
Continental Antarctica							
Queen Maud Land							
Hukuro Cove	1990/1991	12	0.04 ± 0.02	–	–	Yamamoto et al. (1996)	No
Princess Elizabeth Land							
Un-named Island IS 73413*	2019/2020	5	0.25 ± 0.06 (0.19–0.32)	–23.3 ± 1.0 (–24.8, –21.9)	12.0 ± 0.7 (11.3–12.9)	This study	Yes
Magnetic Island	2019/2020	4	0.23 ± 0.03 (0.18–0.25)	–23.4 ± 0.2 (–23.6, –23.2)	11.1 ± 0.2 (10.8–11.3)	This study	Yes
Hop Island	2019/2020	4	0.20 ± 0.08 (0.14–0.32)	–24.3 ± 1.6 (–25.6, –22.2)	10.1 ± 0.8 (9.4–11.1)	This study	Yes
Adélie Land							
Dumont d'Urville	2006/2007	10	0.19 ± 0.06 (0.07–0.27)	–23.5 ± 0.2 (–23.9, –23.2)	10.7 ± 0.4 (10.1–11.3)	Carravieri et al. (2016)	Yes
	2011/2012	10	0.34 ± 0.04 (0.30–0.40)	–22.9 ± 0.5 (–23.2, –21.6)	11.5 ± 0.3 (11.1–12.0)	Carravieri et al. (2016)	Yes
Victoria Land (Ross Sea)							
Edmonson Point	1989-1991	11	0.37 ± 0.15	–	–	Bargagli et al. (1998)	No
Maritime Antarctica							
King George/25 de Mayo Island							
Carlini (Stranger Point)	2019/2020	5	0.12 ± 0.02 (0.09–0.14)	–25.0 ± 0.2 (–25.2, –24.9)	7.6 ± 0.3 (7.2–8.1)	This study	Yes
Hennequin Point + Hannah Point	2013/2014	10	0.10 ± 0.01 (0.07–0.13)	–	–	Souza et al. (2020)	No
Antarctic Peninsula							
Esperanza/Hope Bay	2013/2014	18	0.32 ± 0.14 (0.14–0.63)	–23.7 ± 0.7 (–24.6, –22.0)	8.0 ± 0.5 (7.5–9.7)	McKenzie et al. (2021)	No
	2019/2020	5	0.16 ± 0.01 (0.14–0.18)	–23.5 ± 0.4 (–23.9, –23.0)	7.5 ± 0.3 (7.2–7.8)	This study	Yes
Seymour Island							
Penguin Point	2013/2014	20	0.57 ± 0.20 (0.23–1.05)	–20.0 ± 1.2 (–23.2, –18.9)	11.2 ± 1.1 (7.8–13.4)	McKenzie et al. (2021)	No
Marambio	2019/2020	5	0.23 ± 0.02 (0.19–0.25)	–20.6 ± 0.6 (–21.7, –20.2)	9.1 ± 0.4 (8.6–9.4)	This study	Yes

– No available data (stable isotopes).

*Alpha-numeric identifier in Southwell et al. (2021)

Table 3. AICc model ranking from statistical analyses of feather Hg concentrations from Adélie penguins. Models are Generalized Linear Models (GLMs) with Gamma distribution, and identity (all adults) or inverse (sexed adults and both age classes) link-functions. Abbreviations: k, number of parameters; AICc, Aikaike's Information Criterion adjusted for small sample size; w_i AIC_c weights. Models with ΔAIC_c <2 represent very plausible models (in bold). A model with ΔAIC_{c} = 0 is interpreted as the best model among all the selected ones. Weights are cumulative (sum to 1)

Models	k	AIC_c	ΔAIC_c	w_i
(1) All adults (n=485)				
$\delta^{13}C$ + $\delta^{15}N$ + Colony	**27**	**–596.6**	**0.00**	**1.00**
$\delta^{15}N$ + Colony	26	–574.6	21.94	0.00
$\delta^{13}C$ + Colony	26	–538.8	57.94	0.00
Colony	25	–534.1	62.48	0.00
$\delta^{15}N$	3	–393.8	202.73	0.00
$\delta^{13}C$	3	–204.8	391.79	0.00
NULL	2	–181.0	415.54	0.00
(2) Sexed adults (n=231)*				
$\delta^{13}C$ + $\delta^{15}N$ + Colony	**11**	**–258.4**	**0.00**	**0.58**
$\delta^{13}C$ + $\delta^{15}N$ + Colony + Sex	**12**	**–257.2**	**1.20**	**0.32**
$\delta^{15}N$ + Colony	10	–253.8	4.54	0.06
$\delta^{15}N$ + Colony + Sex	11	–253.2	5.13	0.04
$\delta^{13}C$ + $\delta^{15}N$ + Colony * Sex	19	–246.6	11.80	0.00
$\delta^{15}N$ + Colony * Sex	18	–242.5	15.90	0.00
Colony + Sex	10	–236.5	21.89	0.00
Colony	9	–235.4	22.92	0.00
$\delta^{13}C$ + Colony + Sex	11	–234.4	23.95	0.00
$\delta^{13}C$ + Colony	10	–233.4	24.94	0.00
(3) Adults and pre-fledging chicks from the same colony (n=113)*				
$\delta^{13}C$ + $\delta^{15}N$ + Colony * Age class	**19**	**–232.7**	**0.00**	**0.97**
$\delta^{15}N$ + Colony * Age class	18	–226.0	6.69	0.03
$\delta^{13}C$ + Colony *Age class	18	–213.5	19.17	0.00
Colony * Age class	17	–213.4.	19.28	0.00
$\delta^{13}C$ + $\delta^{15}N$ + Age class	5	–200.9	31.80	0.00
$\delta^{15}N$ + Age class	4	–200.8	31.86	0.00
$\delta^{15}N$ + Colony + Age class	11	–196.7	35.98	0.00
$\delta^{13}C$ + $\delta^{15}N$ + Colony + Age class	12	–194.6	38.07	0.00
Colony + Age class	10	–174.5	58.20	0.00
$\delta^{13}C$ + Colony + Age class	11	–173.0	59.71	0.00

* Only the first 10 models are showed in this table.

Table 4. Estimated parameters of variables included in the best model for each dataset: (1) adults (n=485), (2) sexed adults (n=231) and (3) both age classes (*i.e.,* adults and pre-fledging chicks from the same colony; n=113). Values are estimates [CI] ± SE. Models are Generalized Linear Models (GLMs) with Gamma distribution and identity or inverse link-function. Results are on the response scale for (1) and the inverse scale for (2) and (3). McFadden's R^2 indicates the degree of model fit (*i.e.,* from low and high model fit indicated from 0 to 1, respectively). Feather $\delta^{13}C$ and $\delta^{15}N$ values are proxies of the penguin feeding habitat and trophic position, respectively. When specified, the «+» symbol indicates when the colony (categorical factor) is included in the best model for each dataset. Results for site comparisons (estimated marginal means) are provided in **Table 5** (all adults), **Table S6** (sexed adults) and **Table 6** (both age classes). Abbreviations: CI, confidence interval (95%); SE, standard error.

	Datasets		
	(1) Adults	**(2) Sexed adults**	**(3) Both age classes**
Model specification			
Distribution	Gamma	Gamma	Gamma
Link-function	Identity	Inverse	Inverse
Variables			
Intercept	–1.15 [–1.58, –0.72] ± 0.21	8.42 [4.87, 11.97] ± 1.81	16.28 [9.66–23.02] ± 3.41
$\delta^{15}N$	0.09 [0.06–0.11] ± 0.01	–0.29 [–0.40, –0.17] ± 0.06	–0.58 [–0.84, –0.33] ± 0.13
$\delta^{13}C$	–0.03 [–0.05, –0.02] ± 0.006	0.14 [0.03, 0.26] ± 0.06	0.33 [0.11–0.56] ± 0.11
Colony	+	+	+
Age class			2.49 [1.45–3.60] ± 0.55
McFadden's R^2	0.61	0.67	0.79

Table 5. Estimated marginal mean (EMM) feather Hg concentrations for adult Adélie penguins (n=485) from 24 Antarctic colonies. Estimates were derived from the best-ranked generalized linear model (GLM with Gamma distribution and identity link-function) defined as follows: Hg ~ $\delta^{13}C$ + $\delta^{15}N$ + Colony (see **Table 3** for further details). Abbreviations: CI, confidence interval; SE, standard error.

			95% CI		
Site	**EMM**	**SE**	**Lower**	**Upper**	**Group**
Hukuro Cove	0.46	0.029	0.41	0.52	A
Welch Island	0.37	0.033	0.30	0.43	A
Macey Island	0.52	0.052	0.42	0.62	AB
Un-named Island*	0.39	0.065	0.26	0.52	A
Magnetic Island	0.46	0.071	0.32	0.60	AB
Hop Island	0.32	0.049	0.23	0.42	A
Shirley Island	0.41	0.021	0.37	0.46	A
Dumont d'Urville	0.45	0.026	0.40	0.50	A
Cape Bienvenue	0.46	0.037	0.38	0.53	A
Cape Jules	0.44	0.036	0.37	0.51	A
Adélie Cove	0.78	0.077	0.63	0.93	B
Edmonson Point	0.73	0.070	0.60	0.87	B
Inexpressible Island	0.69	0.068	0.56	0.82	AB
Cape Crozier	0.55	0.052	0.45	0.66	AB
Cape Royds	0.70	0.066	0.57	0.83	AB
Admiralty Bay	0.39	0.012	0.37	0.41	A
Carlini	0.41	0.039	0.33	0.49	A
Ardley Island	0.32	0.031	0.26	0.38	A
Brown Bluffs	0.35	0.029	0.30	0.41	A
Esperanza/Hope Bay	0.48	0.039	0.40	0.55	AB
Marambio	0.53	0.044	0.44	0.61	A
Madder Cliffs	0.29	0.023	0.25	0.34	AC
Paulet Island	0.27	0.021	0.23	0.31	AC
Signy Island	0.34	0.021	0.30	0.38	A

Differences were considered significant when confidence intervals of each colony did not overlap with those of others. Colonies sharing the same Group letters were not significantly different from each other. Colonies with two letters were not significantly different from either group.

* Un-named Island refers to Un-named Island IS 73413*

Table 6. Estimated marginal mean (EMM) feather Hg concentrations for adults and pre-fledging chicks of Adélie penguins from the same Antarctic colonies (n=113). Estimates were derived from the best-ranked generalized linear model (GLM with Gamma distribution and inverse link-function) defined as follows: Hg ~ $\delta^{13}C$ + $\delta^{15}N$ + Colony * Age class (see **Table 3** for further details). Abbreviations: CI, confidence interval; SE, standard error.

Age class	**Adults**				**Chicks**				
			95% CI				**95% CI**		**Age class**
Colonies	**EMM**	**SE**	**Lower**	**Upper**	**EMM**	**SE**	**Lower**	**Upper**	**Difference**
Un-named Island*	0.38	0.037	0.32	0.47	0.19	0.019	0.16	0.24	yes
Magnetic Island	0.44	0.044	0.36	0.54	0.20	0.022	0.16	0.25	yes
Hop Island	0.33	0.033	0.27	0.41	0.19	0.0208	0.15	0.24	yes
Dumont d'Urville 1	0.52	0.044	0.45	0.63	0.17	0.013	0.15	0.20	yes
Dumont d'Urville 2	0.30	0.024	0.26	0.36	0.27	0.023	0.24	0.33	no
Carlini	0.39	0.041	0.32	0.49	0.13	0.016	0.10	0.17	yes
Esperanza/Hope Bay	0.52	0.070	0.41	0.71	0.20	0.031	0.15	0.29	yes
Marambio	0.65	0.11	0.49	0.96	0.34	0.073	0.24	0.59	yes

Differences were considered significant when confidence intervals of adults and chicks from the same colony do not overlap. Dumont d'Urville 1 and 2 refers to samples from 2006/2007 and 2011/2012, respectively.

* Un-named Island refers to Un-named Island* IS 73413*.

Supplementary material

Abbreviations

CO_2	Carbone dioxyde
CuO	Copper oxide
GC	Gaz chromatography
H_2O	Water
Hg	Mercury
N_2	Dinitrogen
NOx	Nitrogen oxydes
RM	Reference material
SSH	Santrock, Studley and Hayes algorithm

Outline

1) Literature review of Hg contamination in penguin feathers from the Southern Ocean

Table S1. Review of feather Hg concentrations in eight penguin species across the Southern Ocean, including both the Subantarctic and Antarctic Zones

2) Material and methods

a. Feather sampling

Table S2. Geographical coordinates (*i.e.*, degrees minutes seconds and decimals) of sampling colonies (*i.e.*, n=24) of Adélie penguins (body feathers) around Antarctica

b. Mercury analyses

Table S3. Certified reference materials (NRC Canada) used for Hg analyses in feathers of Adélie penguins (*Pygoscelis adeliae*): certified (theoretical) and measured values, and recoveries (calculated as measured/certified value)

c. Stable isotope analyses: detailed methodology

d. Outliers from Hukuro Cove

Table S4. Feather Hg concentrations and stable isotope values in five adult Adélie penguins (*Pygoscelis adeliae*) discarded from analyses because of their outlying isotopic values

e. Visualising the non-breeding distribution of Adélie penguins

Fig.S1. Literature review of non-breeding distributions of Adélie penguins (*Pygoscelis adeliae*) tracked from seven Antarctic colonies

3) Sex differences in Adélie penguins

Table S5. Hg concentrations and stable isotope values from feathers of female and male Adélie penguins (*Pygoscelis adeliae*; adults) collected around Antarctica

Fig. S2. Sex differences in Hg concentrations, and $\delta^{13}C$ and $\delta^{15}N$ values measured in feathers of adult Adélie penguins (*Pygoscelis adeliae*) from eight Antarctic colonies

Table S6. Estimated marginal mean (EMM) feather Hg concentrations for sexed adult Adélie penguins (n=231) from eight Antarctic colonies

1) Literature review of Hg contamination in penguin feathers from the Southern Ocean

Table S1. Review of feather Hg concentrations in adults of eight penguin species across the Southern Ocean, including both the Subantarctic and Antarctic Zones. Species include the Adélie (*Pygoscelis adeliae*), chinstrap (*P. antarcticus)* and gentoo penguins (*P. papua*), the emperor (*Aptenodytes forsteri*) and king penguins (*A. patagonicus*), the macaroni (*Eudyptes chrysolophus*), royal (*E. schlegeli*) and southern rockhopper penguins (*E. chrysocome*). Values are means ± SD with ranges in parentheses.

Zone, Region, Site	Species	Year	n	Feather Hg ($\mu g.g^{-1}$)	References
Subantarctic Zone :					
Falkland/Malvinas Islands					
New Island	Gentoo penguin	2016/2017	20	2.97 ± 0.81 (1.77–4.56)	Furtado et al. (2019)
	Southern rockhopper penguin	2016/2017	20	2.24 ± 0.52 (1.43–3.18)	Furtado et al. (2019)
Beauchene Island	Gentoo penguin	2016/2017	20	3.37 ± 0.91 (1.99–4.96)	Furtado et al. (2019)
	Southern rockhopper penguin	2016/2017	20	4.28 ± 0.83 (2.67–5.79)	Furtado et al. (2019)
Kidney Island	Southern rockhopper penguin	2016/2017	25	2.00 ± 0.47 (1.21–3.37)	Furtado et al. (2019)
Crozet Islands					
Possession Island	King penguin	2001	31	1.98 ± 0.73	Scheifler et al. (2005)
	King penguin	2006/2007	12	2.89 ± 0.73 (2.13–4.47)	Carravieri et al. (2016)
	King penguin	2011/2012	11	2.29 ± 0.70 (1.66–4.14)	Renedo et al. (2017)
	Gentoo penguin	2006/2007	11	5.90 ± 1.91 (3.27–8.16)	Carravieri et al. (2016)
	Gentoo penguin	2011/2012	11	4.33 ± 1.85 (1.55–7.59)	Renedo et al. (2017)
	Macaroni penguin	2006/2007	12	2.48 ± 0.35 (1.82–2.92)	Carravieri et al. (2016)
	Macaroni penguin	2011/2012	10	2.27 ± 0.24 (1.85–2.57)	Renedo et al. (2017)
	Southern rockhopper penguin	2006/2007	12	1.79 ± 0.37 (1.20–2.51)	Carravieri et al. (2016)
	Southern rockhopper penguin	2011/2012	10	1.39 ± 0.24 (1.06–1.69)	Renedo et al. (2017)
Kerguelen Islands					
Baie Larose	Southern rockhopper penguin	2006/2007	10	2.54 ± 0.33 (2.00–3.06)	Carravieri et al. (2013)
Cap Cotter	Macaroni penguin	2006/2007	12	2.24 ± 0.29 (1.87–2.75)	Carravieri et al. (2013)
Cap Ratmanoff	King penguin	2006/2007	12	2.22 ± 0.59 (1.45–3.21)	Carravieri et al. (2013)
Estacade	Gentoo penguin	2006/2007	12	5.85 ± 3.00 (1.28–9.43)	Carravieri et al. (2013)
Penn Island	Gentoo penguin	2006/2007	12	1.44 ± 0.44 (0.77–2.06)	Carravieri et al. (2013)
Mayes	Southern rockhopper penguin	2006/2007	12	1.96 ± 0.41 (1.22–2.62)	Carravieri et al. (2013)
Prince Edward Islands					
Marion Island	King penguin	2011	10	3.00 ± 0.71 (2.23–4.65)	Becker et al. (2016)

	Macaroni penguin	2011	10	3.25 ± 0.64 (2.39-4.60)	Becker et al. (2016)
	Southern rockhopper penguin	2011	10	1.80 ± 0.68 (0.93-2.57)	Becker et al. (2016)
Macquarie Island					
	Gentoo penguin	2002/2003	30	0.23 ± 0.29 (0.01–1.50)	Gilmour et al. (2019)
	King penguin	2002/2003	23	0.04 ± 0.02 (0.01–0.09)	Gilmour et al. (2019)
	Royal penguin	2002/2003	23	0.06 ± 0.04 (0.01–0.13)	Gilmour et al. (2019)
	Southern rockhopper penguin	2002/2003	30	0.29 ± 0.23 (0.03–0.91)	Gilmour et al. (2019)
Northern Antarctic Zone :					
South Georgia/Islas Georgias del sur					
Bird Island	Gentoo penguin	1998	14	0.95 ± 0.85 (0.17–3.06)	Becker et al. (2016)
	Gentoo penguin	2009	55	0.97 ± 0.67 (0.15–3.10)	Pedro et al. (2015)
	Gentoo penguin	2011	29	1.13 ± 0.62 (0.23–2.50)	Pedro et al. (2015)
	Macaroni penguin	1998	20	3.42 ± 0.73 (2.08–4.94)	Becker et al. (2016)
Unknown site	King penguin	2008-2012	10	2.92 ± 0.76	Brasso et al. (2013)
Gold Harbour	Gentoo penguin	2008-2012	20	0.85 ± 0.88	Brasso et al. (2015)
	King penguin	2008-2012	20	3.01 ± 0.79	Brasso et al. (2015)
Continental Antarctica :					
Queen Maud Land					
Rumpa Island	Adélie penguin	1981	10	0.17 ± 0.05 (0.11–0.27)	Honda et al. (1986)
Hukuro Cove	Adélie penguin	1991	10	0.09 ± 0.05	Yamamoto et al. (1996)
Adélie Land					
Dumont d'Urville	Adélie penguin	2006/2007	10	0.66 ± 0.20 (0.41–1.06)	Carravieri et al. (2016)
	Adélie penguin	2011/2012	10	0.40 ± 0.09 (0.25–0.52)	Carravieri et al. (2016)
	Emperor penguin	2006/2007	17	1.77 ± 0.37 (1.05–2.76)	Carravieri et al. (2016)
Victoria Land					
Cape Bird	Adélie penguin	2004–2006	154	0.59 ± 0.02	Pilcher et al. (2020)
Cape Crozier	Emperor penguin	2016	10	1.35 ± 0.06	Pilcher et al. (2020)
Edmonson Point	Adélie penguin	1989–1991	3	0.82 ± 0.13	Bargagli et al. (1998)
	Emperor penguin	1989–1991	3	0.98 ± 0.21	Bargagli et al. (1998)
Maritime Antarctica :					
South Shetland Islands					
King George/25 de Mayo Island					
Admiralty Bay	Adélie penguin	2005/2006	20	0.35 ± 0.13 (0.20–0.90)	Brasso et al. (2015, 2014, 2013)
	Adélie penguin	2006/2007	20	0.25 ± 0.12 (0.16–0.70)	Brasso et al. (2015, 2014, 2013)

	Adélie penguin	2007/2008	20	0.38 ± 0.11 (0.21–0.65)	Brasso et al. (2015, 2014, 2013)
	Adélie penguin	2008/2009	16	0.35 ± 0.08 (0.18–0.47)	Brasso et al. (2015, 2014, 2013)
	Adélie penguin	2009/2010	22	0.35 ± 0.14 (0.18–0.69)	Brasso et al. (2015, 2014, 2013) ; Polito et al. (2016)
	Adélie penguin	2010/2011	22	0.35 ± 0.14 (0.18–0.69)	Brasso et al. (2015, 2014, 2013)
	Adélie penguin	2005–2011	120	0.34 ± 0.13 (0.16–0.90)	Brasso et al. (2015, 2014, 2013)
	Chinstrap penguin	2005/2006	9	0.60 ± 0.14 (0.37–0.76)	Brasso et al. (2015, 2014, 2013)
	Chinstrap penguin	2006/2007	16	0.53 ± 0.19 (0.24–0.94)	Brasso et al. (2015, 2014, 2013)
	Chinstrap penguin	2007/2008	9	0.62 ± 0.22 (0.40–1.01)	Brasso et al. (2015, 2014, 2013)
	Chinstrap penguin	2008/2009	16	0.62 ± 0.30 (0.20–1.32)	Brasso et al. (2015, 2014, 2013)
	Chinstrap penguin	2009/2010	20	0.74 ± 0.24 (0.40–1.29)	Brasso et al. (2015, 2014, 2013) ; Polito et al. (2016)
	Chinstrap penguin	2010/2011	20	0.74 ± 0.24 (0.40–1.29)	Brasso et al. (2015, 2014, 2013)
	Chinstrap penguin	2005–2011	90	0.66 ± 0.24 (0.20–1.32)	Brasso et al. (2015, 2014, 2013) ; Polito et al. (2016)
	Gentoo penguin	2005/2006	17	0.35 ± 0.21 (0.14–0.80)	Brasso et al. (2015, 2014, 2013)
	Gentoo penguin	2006/2007	17	0.33 ± 0.21 (0.14–0.85)	Brasso et al. (2015, 2014, 2013)
	Gentoo penguin	2007/2008	17	0.46 ± 0.47 (0.15–2.08)	Brasso et al. (2015, 2014, 2013)
	Gentoo penguin	2008/2009	20	0.41 ± 0.48 (0.13–2.39)	Brasso et al. (2015, 2014, 2013)
	Gentoo penguin	2009/2010	18	0.54 ± 0.55 (0.18–1.98)	Brasso et al. (2015, 2014, 2013) ; Polito et al. (2016)
	Gentoo penguin	2010/2011	21	0.51 ± 0.51 (0.18–1.98)	Brasso et al. (2015, 2014, 2013)
	Gentoo penguin	2005–2011	110	0.44 ± 0.43(0.13–2.39)	Brasso et al. (2015, 2014, 2013) ; Polito et al. (2016)
Narębski Point	Chinstrap penguin	2015/2016	30	0.71 ± 0.34	Catán et al. (2017)
Livingston Island					
Cape Shirreff	Chinstrap penguin	2012	16	1.53 ± 0.08	Álvarez-Varas et al. (2018)
Byers Peninsula	Chinstrap penguin	2008/2009	10	0.40 ± 0.23 (0.20–0.85)	Becker et al. (2016)
	Gentoo penguin	2008/2009	10	0.31 ± 0.14 (0.11–0.63)	Becker et al. (2016)
Hannah Point	Chinstrap penguin	2010/2011	30	0.67 ± 0.46 (0.24–1.57)	Matias et al. (2022)
	Gentoo penguin	2010/2011	30	0.22 ± 0.09 (0.11–0.38)	Matias et al. (2022)
King George and Livingston Islands (combined)					
Hennequin + Hannah Points	Adélie penguin	2013/2014	31	0.40 ± 0.13 (0.16–0.69)	Souza et al. (2020)
	Chinstrap penguin	2013/2014	40	0.63 ± 0.36 (0.16–1.65)	Souza et al. (2020)
	Gentoo penguin	2013/2014	45	0.32 ± 0.13 (0.17–0.91)	Souza et al. (2020)

2) Material and methods

a. Feather sampling

Table S2. Geographical coordinates (*i.e.*, degrees minutes seconds and decimals) of sampling colonies (*i.e.*, 12 sites and 24 colonies) of Adélie penguins (body feathers) around Antarctica. Collaborators indicates principal investigators for each site and colony who contributed to the present study with sampling and/or raw data.

Site, colony	Coordinates	Latitude	Longitude	Collaborators
Continental Antarctica				
Queen Maud Land, Hukuro Cove	69° 12′ 36″ S, 39° 37′ 48″ E	–69.21	39.63	Akinori Takahashi (Syowa Station)
Mac.Robertson Land	67° 36′ 09″ S, 62° 52′ 25″ E	–67.60	62.87	Louise Emmerson, Colin Southwell (Mawson Station)
Welch Island	67° 33′ 36″ S, 62° 55′ 41″ E	–67.56	62.93	
Macey Island	67° 26′ 21″ S, 63° 49′ 09″ E	–67.44	63.82	
Princess Elizabeth Land	68° 34′ 36″ S, 77° 58′ 03″ E	–68.58	77.97	Louise Emmerson, Colin Southwell (Davis Station)
Hop Island	68° 49′ 43″ S, 77° 42′ 08″ E	–68.83	77.70	
Magnetic Island	68° 32′ 35″ S, 77° 54′ 32″ E	–68.54	77.91	
Un-named Island 73413*	68° 27′ 30″ S, 78°22′ 24″ E	–68.46	78.37	
Wilkes Land, Shirley Island	66° 16′ 57″ S, 110° 31′ 36″ E	–66.29	110.53	Louise Emmerson, Colin Southwell (Casey Station)
Adélie Land				Thierry Raclot, Candice Michelot, Akiko Kato
Dumont d'Urville	66° 39′ 49″ S, 140° 0′ 4″ E	–66.67	140.0	
Cape Bienvenue	66° 43′ 14″ S, 140° 31′ 34″ E	–66.72	140.53	
Cape Jules	66° 44′ 30″ S, 140° 55′ E	–66.74	140.92	
Victoria Land				
Edmonson Point	74° 20′ S, 165° 08′ E	–74.33	165.13	Silvia Olmastroni, Ilaria Corsi (Terra Nova Bay)
Adélie Cove	74° 46′ S, 164° 00′ E	–74.77	164.0	
Inexpressible Island	74° 54′ S, 163° 39′ E	–74.90	163.65	
Cape Crozier	77° 31′ S, 169° 24′ E	–77.52	169.40	Annie Schmidt (Ross Island)
Cape Royds	77° 33′ 11″ S, 166° 9′ 49″ E	–77.55	166.16	
Maritime Antarctica				
King George/25 de Mayo Island				
Ardley Island	62° 12′ 45″ S, 58° 55′ 56″ W	–62.21	–58.93	Alvaro Soutello, Ana Laura Machado
Admiralty Bay	62° 10′ 34″ E, 58° 26′ 46 W	–62.18	–58.45	Michael Polito, Rebecka Brasso
Carlini (Stranger Point)	62° 14′ 17″ S, 58° 40′ 1″ W	–62.24	–58.67	Mercedes Santos, Mariana Juáres
Antarctic Peninsula				
Esperanza/Hope Bay	63° 23′ 53″ S, 56° 59′ 47″ W	–63.40	–56.99	Mercedes Santos, Mariana Juáres
Brown Bluff	63° 32′ 0″ S, 56° 55′ 0″ W	–63.53	–56.92	Tom Hart

Seymour Island, Marambio	64° 14′ 31″ S, 56° 37′ 33″ W	–64.24	–56.63	Mercedes Santos, Mariana Juáres
Joinville Island				Tom Hart
Madder Cliffs	63° 18′ 0″ S, 56° 29′ 0″ W	–63.30	–56.48	
Paulet Island	63° 35′ 0″ S, 55° 47′ 0″ W	–63.58	–55.78	
Signy Island, Gourlay	60° 43′ 30″ S, 45° 35′ 17″ W	–60.73	–45.59	Michael Dunn

*Alpha-numeric identifier in Southwell et al. (2021)

b. Mercury analyses

Table S3. Certified reference materials (NRC Canada) used for Hg analyses in feathers of Adélie penguins (*Pygoscelis adeliae*): certified (theoretical) and measured values, and recoveries (calculated as measured/certified value). n indicates sample size for each reference material and values are means ± SD with ranges in parentheses.

Reference material	n	Certified value (µg/g dw)	Measured value (µg/g dw)	Recovery (%)
TORT-3 *Lobster hepatopancreas*	33	0.29 ± 0.02 (0.27 – 0.31)	0.29 ± 0.01 (0.27 – 0.31)	100.6 ± 2.3 (93.6 – 104.7)
DOLT-5 *Fish liver*	21	0.44 ± 0.18 (0.26 – 0.62)	0.43 ± 0.01 (0.41 – 0.46)	96.7 ± 2.6 (93.5 – 105.5)

c) Stable isotopes analyses: detailed methodology

Analytical procedure:

Aliquots were placed in tin capsules (8 mm x 5mm, Elemental Microanalysis Ltd, Okehampton, United Kingdom) and analyzed for carbon and nitrogen stable isotope compositions, using a continuous-flow system consisting of an elemental analyzer (Flash 2000, or Flash IRMS EA Isolink CN, Thermo Scientific, Milan, Italy) equipped with the smart EA option and an autosampler (Zero Blank, Costech, Valencia, CA, United States). The elemental analyzer was connected via a Conflo IV peripheral to a Delta V Plus isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany). In the elemental analyzer, each sample was combusted quantitatively in a first reactor containing copper oxide (Elemental Microanalysis Ltd, Okehampton, United Kingdom) and silvered cobaltous oxide (Elemental Microanalysis Ltd, Okehampton, United Kingdom). Oxygen (grade 99.9999% purity; Air Liquide, France) was pulsed during combustion and helium was used as carrier gas (grade 99.9999% purity, 100 ml.min-1; Air Liquide, France). Gases resulting from the combustion were flushed in a second reactor where NOx gases were reduced at 650°C using copper wires (Elemental Microanalysis Ltd, Okehampton, United Kingdom) to produce pure N_2. Gases obtained (CO_2, N_2 and H_2O) were carried through a water trap (granular magnesium perchlorate, Elemental Microanalysis Ltd, Okehampton, United Kingdom) and then a GC column. N_2 and CO_2 were then introduced into the isotope ratio mass spectrometer. The instrumentation was controlled and measurement data were obtained using the instrumental software Isodat 3.0 (Thermo Scientific, Bremen, Germany).

Metrological traceability:

Results are reported in per mil (‰) using the delta (δ) notation and were normalized using reference materials: Vienna Pee Dee Belemnite (VPDB) and atmospheric nitrogen (Air-N_2) for carbon and nitrogen, respectively. Normalization was carried out using USGS61 and USG63 (US Geological Survey, Reston, VA, USA) based on their assigned carbon and nitrogen isotope- δ values and standard uncertainties (*i.e.*, −35.05 ± 0.04 ‰ and −1.17 ± 0.04 ‰ for carbon, respectively, and −2.87 ± 0.04 ‰ and +37.83 ± 0.06 ‰ for nitrogen, respectively). Each reference material was measured in duplicate at the beginning and the end of each daily group of analysis.

Data processing: Isotope- δ values for the CO_2 and N_2 gas derived from the samples relative to a working gas were obtained using the instrumental software (Isodat 3.0, Thermo Scientific, Bremen, Germany). Working gases were introduced from high-pressure cylinders (CO_2 99.998% purity and N_2 99.9999% purity; Air Liquide, France) directly into the mass spectrometer. Blank correction was done for both CO_2 and N_2, and "SSH" ^{17}O correction was applied for CO_2. A drift correction was applied using the results of reference materials analyzed regularly (*i.e.*, after every 20 or 21 sample measurements) in each analytical batch. Special care was taken to ensure that the amount of yield gas was the same for reference materials and samples, but minor differences were normalized using the signal amplitude of the reference materials ran in each analytical batch. The $\delta^{13}C$ and $\delta^{15}N$ values were normalized by two-point normalization. All corrections and normalizations were performed simultaneously using multiple linear least square regression.

Uncertainty evaluation: The uncertainty of the reported isotope- δ values was evaluated as the standard deviation of repeated (n = 5 or 8) measurements of each reference material *(i.e.*, USGS61 and USGS63) within a single group of analyses. Uncertainty did not exceed 0.10 ‰ for both $\delta^{13}C$ and $\delta^{15}N$ values.

d) Outliers from Hukuro Cove

Table S4. Feather Hg concentrations and stable isotope values in five adult Adélie penguins (*Pygoscelis adeliae*) discarded from the analyses because of their outlying high isotopic values ($\delta^{13}C$ and $\delta^{15}N$ values are proxies of penguin feeding habitat and trophic position, respectively).

Site, Colony	ID	Feather Hg (µg·g^{-1} dw)	Feather $\delta^{13}C$ (‰)	Feather $\delta^{15}N$ (‰)
Syowa				
Hukuro Cove	10a	0.67	–20.7	13.2
Hukuro Cove	63b	0.92	–19.0	14.5
Hukuro Cove	65b	0.82	–20.1	13.1
Antarctic Peninsula				
Brown Bluffs	9	0.83	–18.8	11.1
Brown Bluffs	11	1.22	–17.7	13.1

e) Visually representing the non-breeding distribution of Adélie penguins

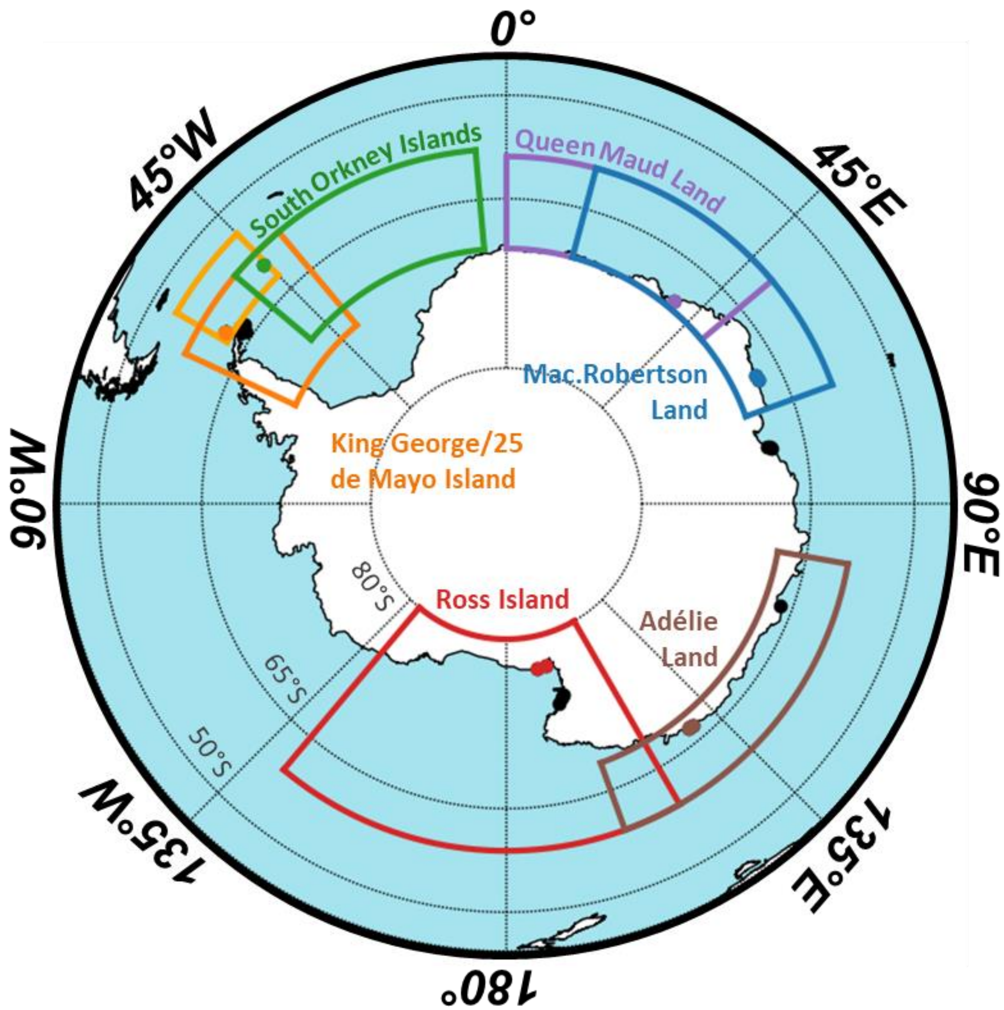

Figure S1. Literature review of non-breeding distributions of Adélie penguins (*Pygoscelis adeliae*) tracked in seven Antarctic sites: South Orkney Islands (Signy Island, Dunn et al., 2011; in green), Queen Maud Land (Hukuro Cove, Takahashi et al., 2018; in purple), Mac.Robertson Land (Mawson Station, Béchervaise Island, Clarke et al., 2003; in blue), Adélie Land (Dumont d'Urville, Thiébot et al., 2019; in brown), Victoria Land (Ross Sea, Cap Bird, Ballard et al., 2010; Davis et al., 1996; in red) and King George/25 de Mayo Island (Admiralty Bay, Hinke et al., 2015; in dark orange; Ardley Island, Zaldúa et al., unpublished; in light orange). Distributions were defined as the maximum geographical zone including all individual tracks. Black dots represent other sites sampled in the present study (see **Fig.1** and **Table S2** for further details).

3) Sex differences in Adélie penguins

Table S5. Mercury concentrations and stable isotope values from feathers of female and male Adélie penguins (*Pygoscelis adeliae*; adults) collected around Antarctica. n indicates sample sizes. Feather carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) valus are proxies of penguin feeding habitat and trophic position, respectively. Values are means ± SD with ranges in parentheses.

Site, Colony, Sex (Year)	n	Feather Hg (µg.g^{-1} dw)	Feather $\delta^{13}C$ (‰)	Feather $\delta^{15}N$ (‰)
Continental Antarctica				
Queen Maud Land, Hukuro Cove (2010)				
Female	15	0.44 ± 0.09 (0.32–0.66)	–24.5 ± 0.6 (–25.7, –23.5)	9.4 ± 0.5 (8.6–10.4)
Male	12	0.53 ± 0.12 (0.35–0.69)	–23.1 ± 0.8 (–24.9, –22.9)	10.1 ± 0.9 (9.0–12.1)
Adélie Land, Dumont d'Urville (2012)				
Female	3	0.47 ± 0.08 (0.37–0.52)	–24.0 ± 0.7 (–24.6, –23.3)	11.4 ± 0.5 (11.1–11.9)
Male	7	0.37 ± 0.09 (0.25–0.47)	–24.4 ± 0.7 (–25.6, –23.5)	10.6 ± 0.7 (9.8–11.5)
Victoria Land, Terra Nova Bay: (2017)				
Adélie Cove				
Female	8	0.91 ± 0.27 (0.50–1.35)	–24.2 ± 0.9 (–25.3, –22.5)	11.0 ± 1.2 (9.9–12.8)
Male	7	0.94 ± 0.26 (0.49–1.27)	–24.7 ± 0.6 (–25.6, –24.0)	10.9 ± 0.8 (10.1–12.1)
Edmonson Point				
Female	8	0.75 ± 0.12 (0.50–0.93)	–24.8 ± 0.5 (–25.5, –24.1)	10.3 ± 0.5 (9.8–11.0)
Male	7	0.92 ± 0.19 (0.63–1.18)	–24.2 ± 0.8 (–25.8, –23.6)	11.0 ± 0.4 (10.4–11.6)
Inexpressible Island				
Female	7	0.70 ± 0.24 (0.50–1.22)	–24.6 ± 1.0 (–25.6, –22.8)	10.7 ± 1.3 (9.0–12.8)
Male	8	0.95 ± 0.23 (0.66–1.33)	–24.3 ± 0.8 (–25.3, –22.9)	11.2 ± 0.8 (10.1–12.2)
Victoria Land, Ross Island: (2019)				
Cape Crozier				
Female	9	0.58 ± 0.09 (0.45–0.68)	–25.9 ± 0.3 (–26.3, –25.5)	9.7 ± 0.5 (9.1–10.6)
Male	5	0.69 ± 0.15 (0.51–0.92)	–25.9 ± 0.4 (–26.5, –25.4)	9.8 ± 0.7 (9.3–10.9)
Cape Royds				
Female	6	0.78 ± 0.18 (0.61–1.02)	–25.0 ± 1.1 (–25.8, –22.8)	10.1 ± 1.0 (9.2–11.8)
Male	9	0.81 ± 0.20 (0.56–1.08)	–25.7 ± 0.2 (–25.9, –25.3)	10.4 ± 0.7 (9.7–11.9)

Maritime Antarctica				
King George/25 de Mayo Island, Admiralty Bay				
(2005)				
Female	10	0.37 ± 0.21 (0.20–0.90)	–25.0 ± 0.8 (–25.7, –23.3)	8.9± 0.4 (8.3–9.7)
Male	10	0.33 ± 0.08 (0.24–0.52)	–24.8 ± 0.6 (–25.4, –23.5)	8.8 ± 0.3 (8.1–9.2)
(2006)				
Female	9	0.21 ± 0.06 0.16–0.36	25.0 ± 0.3 (–25.5, –24.6)	8.6 ± 0.3 (8.1–8.9)
Male	11	0.29 ± 0.14 (0.20–0.70)	–25.1 ± 0.4 (–25.7, –24.7)	8.8 ± 0.4 (8.3–9.6)
(2007)				
Female	9	0.41 ± 0.06 (0.31–0.47)	–24.9 ± 0.3 (–25.2, –24.3)	9.0 ± 0.2 (8.8–9.2)
Male	11	0.36 ± 0.14 (0.21–0.65)	–24.7 ± 0.5 (–25.3, –23.9)	8.9 ± 0.4 (8.5–9.7)
(2008)				
Female	7	0.37 ± 0.07 (0.27–0.47)	–24.6 ± 0.5 (–25.2, –24.0)	8.8 ± 0.2 (8.5–9.0)
Male	9	0.33 ± 0.09 (0.18–0.46)	–24.6 ± 0.6 (–25.3, –23.8)	9.0 ± 0.6 (8.3–10.5)
(2009)				
Female	11	0.36 ± 0.09 (0.19–0.49)	–24.1 ± 0.9 (–25.4, –22.9)	9.0 ± 0.4 (8.0–9.6)
Male	11	0.34 ± 0.18 (0.18–0.69)	–23.8 ± 0.8 (–25.0, –22.4)	9.1 ± 0.4 (8.6–10.1)
(2010)				
Female	11	0.36 ± 0.09 (0.19–0.49)	–24.4 ± 1.3 (–27.4, –22.9)	9.0 ± 0.4 (8.0–9.6)
Male	11	0.34 ± 0.18 (0.18–0.69)	–23.8 ± 0.8 (–25.0, –22.4)	9.1 ± 0.4 (8.6–10.1)

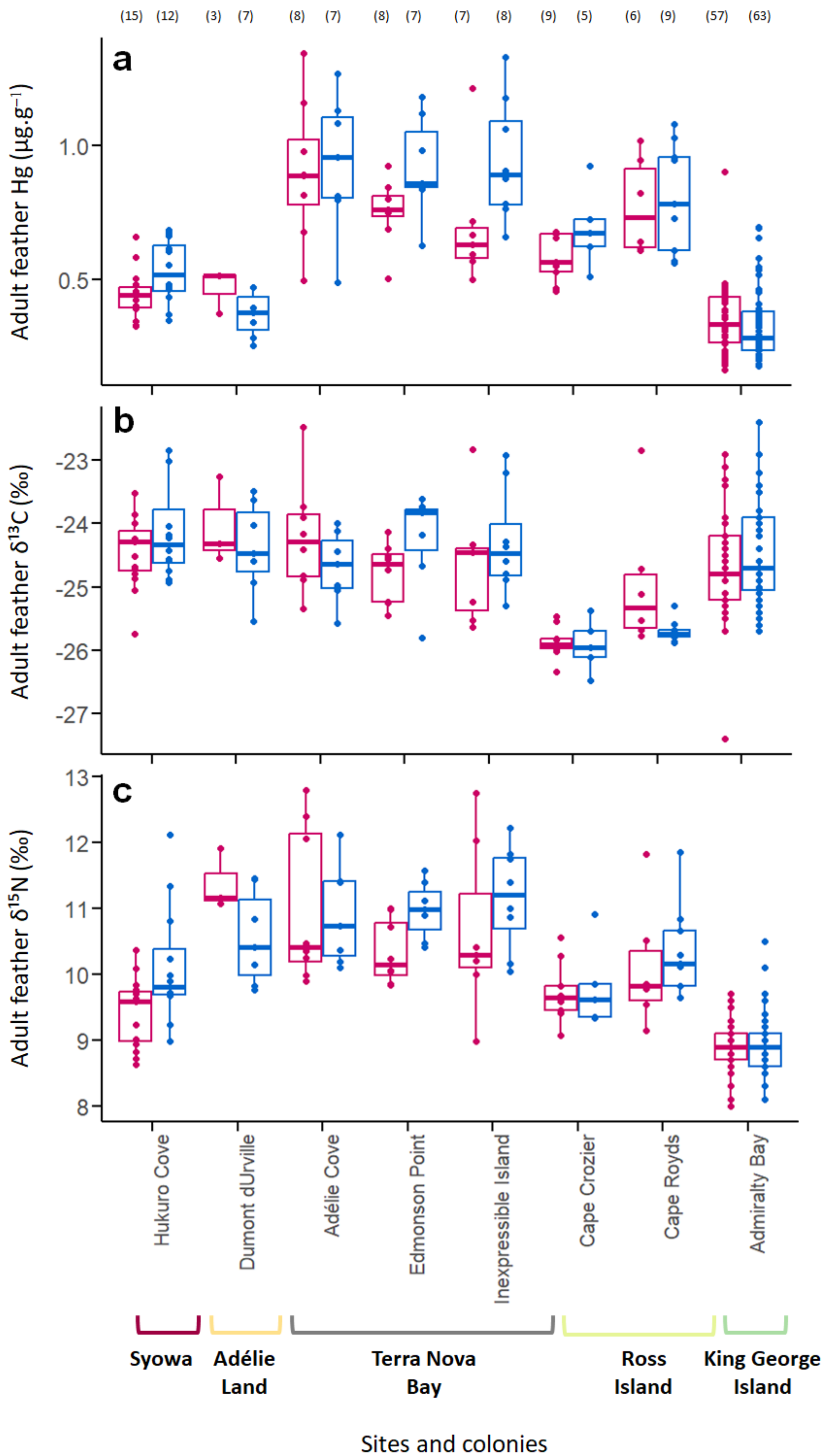

Figure S2. Sex differences in **(a)** Hg concentrations, and **(b)** $\delta^{13}C$ and **(c)** $\delta^{15}N$ values measured in feathers of adult Adélie penguins (*Pygoscelis adeliae*) from eight Antarctic colonies. Females and males are represented in pink and blue, respectively. Feather $\delta^{13}C$ and $\delta^{15}N$ values are proxies for penguin feeding habitat and trophic position, respectively. Numbers in brackets represent sample sizes for each sex and colony. Individual values (smaller dots) are presented with boxplots, representing median values (midlines), errors bars (whiskers) and outliers (black dots outside whiskers)

Table S6. Estimated marginal mean (EMM) feather Hg concentrations for sexed adult Adélie penguins (n=231) from eight Antarctic colonies. Estimates were derived from the best-ranked generalized linear model (GLM with Gamma distribution and inverse link-function) defined as follows: Hg ~ $\delta^{13}C$ + $\delta^{15}N$ + Colony (see **Table 3** for further details). Abbreviations: CI, confidence interval; SE, standard error.

			95% CI		
Sites	**EMM**	**SE**	**Lower**	**Upper**	**Group**
Hukuro Cove	0.48	0.029	0.43	0.55	A
Dumont d'Urville	0.35	0.032	0.30	0.43	ABD
Adélie Cove	0.67	0.055	0.58	0.80	C
Edmonson Point	0.67	0.051	0.58	0.78	C
Inexpressible Island	0.62	0.051	0.53	0.74	C
Cape Crozier	0.54	0.046	0.46	0.65	AC
Cape Royds	0.63	0.052	0.54	0.75	AC
Admiralty Bay	0.37	0.012	0.34	0.39	D

Differences were considered significant when confidence intervals did not overlap. Colonies sharing the same Group letters are not significantly different from each other. Colonies with two letters were not significantly different from either group.

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