





# Latitudinal, sex and inter-specific differences in mercury and other trace metal concentrations in Adélie and Emperor penguins in the Ross Sea, Antarctica

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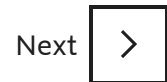
## Highlights

- Hg in Adélie and emperor penguins from the Ross Sea lower than other penguin species
- Female Adélie penguins from Cape Bird had lower Hg concentrations than males.
- Hg was greater in Adélie penguins breeding at higher latitudes
- Hg was higher in emperor penguin than Adélie penguins.
- Cd, Cu and Zn were higher in Adélie penguins compared with emperor penguins.

- $\delta^{15}\text{N}$  in emperor penguins was higher than that for Adélie penguins.

## Abstract

We sought to determine mercury (Hg) and other trace metal concentrations in Adélie (*Pygoscelis adeliae*) and emperor penguin (*Aptenodytes forsteri*) breast feathers from the Ross Sea, Antarctica, and relate those concentrations to the trophic position and the habitats in which each of these species forage. Adélie penguin feathers from the southern Ross Sea colonies were higher in Hg than those sampled further north in the Ross Sea, potentially due to greater exposure to local sources, such as volcanism. Female Adélie penguins had lower feather total Hg concentrations than males. This may reflect female penguin's capacity to eliminate Hg through the egg development and laying process, or the larger and/or older prey items that male birds can consume, reflected by their higher trophic position. Emperor penguins have higher Hg concentrations than Adélie penguins which is also partially explained by Adélie penguins feeding at lower trophic levels than emperor penguins.



## Keywords

Adélie penguins; Contaminants; Emperor penguins; Ross Sea; Stable isotope ratios; Trace elements

## 1. Introduction

The presence of pollutants in the form of trace metals, such as mercury (Hg), in polar marine environments has been documented for decades (e.g., [Muir et al., 1992](#); [Dietz et al., 1995](#); [Atwell et al., 1998](#)) and high latitude systems are considered a sink in the global Hg cycle ([Ariya et al., 2004](#); [Pfaffhuber et al., 2012](#)). Mercury is a naturally occurring element which can be released into the wider environment by natural geophysical processes or anthropogenic activities. Sources of Hg from geophysical processes include erosion, flooding, upwelling, and volcanic emissions (e.g. Mount Erebus; [Bargagli et al., 1998](#); [Burger and Gochfeld, 2004](#)), while anthropogenic sources include industrial processes, and agricultural practices ([Pirrone et al., 2010](#)). Two-thirds of the Hg in the atmosphere is

estimated to originate from human activities such as coal-fired electricity generation and gold mining (Morel et al., 1998; Streets et al., 2009; Pirrone et al., 2010). It is also fairly unique in the biogeochemistry cycle being one of the few metals that volatilises and is transported to polar regions through the 'global distillation' phenomenon (or the 'grasshopper effect'; O'Driscoll et al., 2005).

While evidence has been limited owing to a lack of consistent monitoring (Pirrone et al., 2010; Sprovieri et al., 2010), global increases in atmospheric Hg have been inferred from changes in concentrations over the Atlantic Ocean between 1977 and 1990 (Slemr and Langer, 1992). More recent increases in atmospheric Hg have largely been attributed to emissions from anthropogenic activities, especially from industrial development and an increasing demand for energy from developing countries in Asia, Africa and South America (Bargagli, 2008). In 2013, the United Nations Environment Program (UNEP) enacted the Minamata Convention on Mercury (Larson, 2014) with an aim of initiating international action to manage Hg and protect human health and the environment from the adverse effects of Hg (Kessler, 2013).

Aligned to increases in atmospheric Hg concentrations has been the build-up of Hg in the surface waters of the oceans globally, which have almost tripled in the last 300 years (Lamborg et al., 2014). It is thought that Hg is deposited into the ocean primarily via the atmosphere (Cossa et al., 2011). Deposition can occur by way of Atmospheric Mercury Depletion Events (AMDE) that comprise a rapid oxidation and deposition of Hg from the atmosphere during the onset of a polar spring (Schroeder et al., 1998). AMDE are photochemical reactions that occur during spring after sunrise in high latitude environments, like Antarctica (Ebinghaus et al., 2002). Antarctic waters therefore have some of the highest methylmercury concentrations in the world's open oceans (Cossa et al., 2011).

Seabirds are top predators and therefore act as useful bioindicators of pollutants in the environment (Burger and Gochfeld, 2002; Bond and Lavers, 2011). It is well established that Hg can cause developmental, neurological, behavioural and physiological impairments in a wide range of wildlife species (Wolfe et al., 1998; Spalding et al., 2000; Goutte et al., 2014a, Goutte et al., 2014b) and act as an endocrine disruptor (Tartu et al., 2013). In birds, some metals can adversely affect mobility and balance and cause an increase in mortality (Newman, 2015).

As long-lived meso-predators that tend to return to annual breeding colonies, Antarctic penguins (Family Spheniscidae) such as Adélie (*Pygoscelis adeliae*) and emperor penguins (*Aptenodytes forsteri*), are potentially useful sentinels of pollution at a regional scale (Espejo et al., 2014). Emperor penguins reportedly travel up to ~600km from their breeding

site to moult ([Wienecke et al., 2004](#)), while Adélie penguins that breed on Ross Island in the southern Ross Sea can travel an estimated 1800km to overwinter near the Antarctic circle ([Ballard et al., 2010](#)). In comparison, some flying seabirds have extensive home ranges (e.g., the trans-equatorial migrant, the sooty shearwater, *Puffinus griseus*, covers approx. 64,000km in a roundtrip of the Pacific Ocean; [Shaffer et al., 2006](#)) which can render tracing the source of Hg and other trace metals difficult.

The Western Ross Sea alone is home to about 1.7 million breeding Adélie penguins ([Lyver et al., 2014](#)) and at least 60,000 emperor penguins ([Fretwell et al., 2012](#)). Adélie (4.07±0.08kg; [Cockrem et al., 2006](#)) and emperor penguins (38.2±0.7kg; [Groscolas, 1986](#)) are considered to be medium and large size penguins respectively, both with lifespans of more than ten years ([Brasso et al., 2014](#)). Both emperor and Adélie penguin individuals replace their plumage completely on an annual basis ([Carravieri et al., 2014b](#)), which allows for a more accurate assessment of Hg body burden over time ([Carravieri et al., 2014a](#)). Most seabirds moult their feathers sequentially and those feathers produced earlier in the moulting period contain more Hg than those produced later ([Bearhop et al., 2000](#)). In contrast, penguin feathers are grown simultaneously and so provide a less variable representation of Hg concentration and isotopic value ([Carravieri et al., 2014a](#)). Adélie penguins are also thought to have a similar diet year-round ([Brasso et al., 2014](#)) which further lends support to their use as a sentinel of Hg and other trace metals in the environment. A consistent intra-annual diet helps to alleviate concerns about the validity of using feathers to assess the relationship between Hg concentrations and stable isotopic proxies of trophic position, given that the former is accumulated in the body over a longer time period ([Carravieri et al., 2013](#)).

Feathers are well suited for Hg analysis because they are both chemically and physically stable ([Monteiro and Furness, 1995](#)) and are the predominant mechanism by which birds eliminate Hg ([Braune and Gaskin, 1987](#)). Feathers are a sensitive indicator of Hg burden because this trace metal has a particular affinity for keratin owing to keratin's high proportion of sulphur amino acids ([Block, 1951](#)). Stable isotope analysis using feathers provides information about diet over the period that the feathers are grown prior to the moult stage which is a longer period than other tissues such as blood ([Bearhop et al., 2000](#)). The sex and breeding status of individuals may also affect the stable isotopic composition of blood ([Bearhop et al., 2002](#)), but feathers are thought to be unaffected by this ([Labbe et al., 2013](#)).

The levels of Hg and other trace metals in seabirds are determined by their dietary intake ([Lock et al., 1992](#)), and information about trophic level may assist with the interpretation of

Hg cycling (Bearhop et al., 2000) as Hg generally increases with trophic level (Aronson et al., 2011). For example, it can help to determine the sources at the base of the food web (Kelly, 2000) and distinguish whether changes in Hg concentrations observed over time are attributable to changes in the concentration of that trace metal in the environment, or changes to diet composition (Furness et al., 1995).

Stable nitrogen and carbon isotope ratios provide information about average diet over time, in terms of both trophic levels and prey habitat, respectively (Bond and Jones, 2009).

Penguin diet reconstruction by stable isotope analysis requires data of the isotopic composition of their prey (Post, 2002). The abundance of nitrogen isotopes in an organism depends on the nitrogen isotopes in its diet (Deniro and Epstein, 1981). Organisms have higher  $\delta^{15}\text{N}$  values than their prey because  $^{15}\text{N}$  is preferentially fractionated into body tissues in comparison to  $^{14}\text{N}$  (Minagawa and Wada, 1984). For example, krill, squid, and fish have both distinct trophic levels and distinct  $\delta^{15}\text{N}$  values (Zimmer et al., 2007).

Determining carbon isotope compositions can aid in understanding the type of habitat an individual forages in. For example, organisms at the base of the food web that live in sympagic (ice-associated) environments, such as ice algae, tend to be more enriched in  $^{13}\text{C}$  than organisms that live in pelagic (open sea) environments, such as phytoplankton (Søreide et al., 2006) due to differences in how dissolved inorganic carbon is fixed in these distinct marine habitats. Habitat-specific enrichment in  $^{13}\text{C}$  is passed up the food web, therefore stable carbon isotope analysis can identify the extent to which ice algae or phytoplankton form the basis of a species' assimilated diet. (Hobson et al., 1995). An analysis of stable carbon isotopes in Antarctic penguins can act like a tracer to provide information about the habitat of its prey and primary producers at the base of the food web (Hobson et al., 1994).

In this study, feathers were used to assess total Hg concentrations of Adélie penguins in the Ross Sea over time, among different age groups, between the sexes, and latitudinal groupings (relatively high latitude of Cape Bird versus relatively low latitude of Cape Hallett and Cape Adare). The concentrations of total Hg and other trace metals [arsenic (As), cadmium (Cd), copper (Cu), lead (Pb) and zinc Zn] were also compared between emperor and Adélie penguins. Feather nitrogen and carbon stable isotope ratios were used to determine trophic position and foraging habitat respectively.

## 2. Methods

### 2.1. Field sampling

Adélie penguin feathers were collected from breeding adults at Cape Bird, Ross Island (2004–2007, 2009–2010, 2012, 2014 and 2016 inclusively) and Cape Hallett (2005) and Cape Adare (2015), Victoria Land (Table 1). Adult emperor penguin feathers were collected at Cape Crozier, Ross Island in 2016. Six feathers were plucked from the breast area of each sampled individual. To identify individuals and avoid repeat sampling, a semi-permanent blue or red dye (CeeMark stock marker) was used to paint a patch of each bird's breast area before release. Researchers wore a fresh pair of non-powdered nitrile gloves for each individual Adélie penguin but bare fingers for emperor penguin feather plucking because the gloves impeded grip. Feathers were stored in plastic zip lock bags and labelled externally. Once in New Zealand, all feather samples were stored in a freezer ( $-20^{\circ}\text{C}$ ) in the laboratory.

Table 1. Number of Adélie penguins (*Pygoscelis adeliae*) sampled across year, age and sex at the Cape Bird, Cape Hallett and Cape Adare breeding colonies in the Ross Sea, Antarctica between 2004 and 2016.

Year	Total birds sampled	Aged	Sexed	Notes
2004	10	0	0	
2005	20	0	5	10 birds were from Cape Hallett
2006	14	0	10	
2007	15	0	15	
2009	17	17	0	
2010	29	1	0	
2012	10	0	0	
2014	10	0	0	
2015	10	0	0	All from Cape Adare
2016	39	29	0	
Total	174	47	30	

## 2.2. Feather analysis

Each feather sample encompassed up to three feathers from a single individual. Feather samples were washed with ultrapure water ( $>18$  mega-ohms) and RBS-35 detergent ( $\leq 1\%$



sodium hydroxide) to remove surface contaminants and then rinsed with de-ionised water. Feathers were then placed into pre-weighed vials and dried overnight at 35 °C before being re-weighed.

The method of sample analysis was modified from [Lyver et al. \(2017\)](#). For each sample, 0.45 mL of ultrapure (70%) nitric acid (HNO<sub>3</sub>) and 0.05 mL of ultrapure (24%) hydrochloric acid (HCl) was pipetted into each vial containing the pre-weighed feathers. These were immediately capped and left overnight to pre-digest. The following day, the samples were heated at 85 °C for 2 h and left to cool overnight, before 2.5 mL of 2% HNO<sub>3</sub>/0.5% HCl/0.1% L-Cysteine (aqua regia) solution was pipetted into each vial. The solution contained cysteine because Hg has a strong affinity for thiol-containing compounds. Cysteine has been shown to decrease the memory effect of Hg ([Li et al., 2006](#)), which can reduce the analytical sensitivity over time. The mass of each acid digest was used to calculate the final volume ([Harrington et al., 2004](#)).

Samples were analysed using an Agilent 7500 ICP-MS series fitted with a collision cell (He gas) to eliminate polyatomic interference [Arsenic (75As); cadmium (111Cd); copper (63Cu); lead (sum of 206Pb, 207Pb, 208Pb); mercury (201Hg) and zinc (66Zn) with Rhodium (108Rh) added on-line as an internal standard]. Calibration standards ranged from 0.1–1000 µg L<sup>-1</sup> and a water Certified Reference Material (CRM; Synthetic 1643 CRM; Inorganic Ventures) was used to confirm the accuracy of the calibration. Replicates ( $n=3$ ) of CRM ERMDB001 Human Hair were digested and analysed with each batch of feather samples. The mean recovery ranged from 73 to 102% for Cd, Cu, Pb and Zn, and was 83% for Hg. Arsenic concentrations in CRM ERMDB001 were below the detection limit.

All feather samples were analysed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  using a Costech Elemental Combustion System (ECS) 4010 (Costech Analytical Technologies, California, USA) connected to a Delta V Plus Isotope Ratio Mass Spectrometer (IRMS; Thermo Fischer Scientific, Massachusetts, USA) via a Finnigan ConFlo III (Thermo Fischer Scientific). All samples were loaded into a ZeroBlank autosampler with an isolation valve (Costech Analytical Technologies) and were individually combusted at 1050 °C under a continuous flow (c. 110 mL min<sup>-1</sup>) of ultra-high-purity helium (>99.999%). The produced N<sub>2</sub> and CO<sub>2</sub> were separated using a gas chromatography column held at a constant 45 °C. IRMS peak jumps were calibrated at least daily, and reference gas linearity tests were performed at the start of every other analytical sequence. Internal precision (i.e., zero-enrichment test) was determined prior to every analytical sequence and was always <0.06‰ for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Data were normalized to V-PDB scale for  $\delta^{13}\text{C}$  and Air for  $\delta^{15}\text{N}$  using a stretch-and-shift 2-point normalization based on replicate analyses of certified reference materials (e.g. IAEA-CH-3; USGS24;

NBS22; IAEA-N-1; IAEA-N-2) within individual analytical sequences. External precision, or the standard deviation of replicate analyses of certified reference materials and internal laboratory check standards over the course of the study, was <0.20% for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .

## 2.3. Sexing Adélie penguins

### 2.3.1. DNA extractions

Genomic DNA was isolated from the tips of 1–3 plucked feathers, using the Qiagen DNeasy Tissue kit (Qiagen) following the user protocol with several modifications. Modifications included digesting samples for 24h in 40  $\mu\text{L}$  Proteinase K and ATL buffer at 56°C, and raising the temperature to 60°C for the final 15 min of the digest, with samples being re-suspended every 30min at 450 RPM on a Thermomixer Comfort (Eppendorf) for 20s. Following digestion, samples were incubated at 70°C in AL buffer for 45 min and resulting DNA was precipitated in cold 100% ethanol. DNA was eluted twice in 100  $\mu\text{L}$  AE buffer, first at 70°C for 15 min, then following an initial centrifugation step the AE buffer was recycled to the spin column membrane and incubated at 70°C for an additional 5 min, before final centrifugation. Resulting DNA was quantified using a high sensitivity Qubit assay kit (ThermoFisher Scientific), and quantity and quality were visualised by running 5  $\mu\text{L}$  DNA on a 2% agarose gel electrophoresis at 75V. Samples that resulted in a low DNA yield <0.50ng/ $\mu\text{L}$  or that did not amplify a product during polymerase chain reaction (PCR) were re-extracted.

### 2.3.2. Polymerase chain reaction

We amplified a 665 and 747 base pair (bp) region of the CHD1 gene region from both the Z and W chromosomes using one set of primers (2550F/2718R) developed by [Zhang et al. \(2013\)](#). PCR's (10  $\mu\text{L}$ ) were performed using 1x PCR Buffer, 250  $\mu\text{M}$  each dNTP, 1.25U i-Taq (iNtRON), 0.25  $\mu\text{M}$  each primer, and 1  $\mu\text{L}$  DNA extract on a BIO-RAD MyCycler thermal cycler with an initial denaturation of 94°C for 5 min, followed by 40 cycles of 94°C for 30s, 55°C for 45s and 72°C for 45s, and a final 10 extension at 72°C for 10 min. Amplified products were visualised on a 3% agarose gel electrophoresis at 85V, using SYBR safe stain, where males were represented with one band, and females with two bands. PCR's were repeated twice to ensure sexing was accurate for each sample.

## 2.4. Data analysis

### 2.4.1. Hg in Adélie penguins



The effects of sex, age, and latitude on Hg concentration in Adélie penguins were assessed in a Bayesian mixed effects model with year specified as a random effect. The effect of sex ( $\beta_{\text{sex}}$ ) was specified as the effect of males relative to females. The effect of latitude ( $\beta_{\text{lat}}$ ) was specified as the effect of sites at higher latitudes (Cape Bird) relative to lower latitudes (Cape Adare and Cape Hallett). Samples from Cape Adare and Cape Hallett were pooled into 'lower latitude' sites due to the relative closeness of locations and the small number of samples ( $n=10$ ) from each of these two colonies.

The concentration of Hg in each individual  $i$  was specified as a random variate from a normal distribution:

$$\text{Hg}_{[i]} \sim \text{Normal}(\mu_{[i]}, \sigma)$$

where

$$\mu_{[i]} = \beta_0 + \beta_{\text{sex}} \times \text{Sex}_{[i]} + \beta_{\text{lat}} \times \text{Latitude}_{[i]} + \beta_{\text{age}} \times \text{Age}_{[i]} + \gamma_{[\text{year}[i]]}$$

Fitting a model with interaction terms was not possible due to the sparseness of some of the covariates: all of the sexed and aged individuals were from the same location (Cape Bird), and none of the sexed individuals were aged or vice versa (see Results summary, [Table 1](#)). The model was fitted using a Bayesian Lasso approach ([Park and Casella, 2008](#)) where all parameters are included in a single model. Prior probabilities on all coefficients were specified as  $\beta_j \sim \text{Dexp}(0, \lambda)$  with a single  $\lambda$  for all coefficients, drawn from a prior probability  $\lambda \sim \text{Uniform}(0.001, 5)$ . For covariates that were not measured in all individuals (e.g. sex and age), missing values were sampled at each iteration;  $\text{Sex}_i \sim \text{Bernoulli}(0.5)$  and  $\text{Age}_i \sim \text{Poisson}(10)$ .

## 2.4.2. Concentration of trace metals in Adélie and Emperor penguins

Concentrations of six trace metals (As, Cd, Cu, Hg, Pb and Zn) were compared separately between Adélie and emperor penguins at high latitude colonies only (Cape Bird and Cape Crozier). We used a Bayesian analogue of a  $t$ -test which provides much richer information than the null-hypothesis significance  $t$ -test ([Kruschke, 2013](#)) and enables the model to be easily extended to include covariates. The concentration of an element in individual  $i$  of species  $s$  was specified as a random variate from a normal distribution:

$$\text{X}_{[i]} \sim \text{Normal}(\mu_{[i]}, \sigma)$$

where

$$\mu_{[i]} = \beta_{[\text{spp}[i]]}$$

where  $s=1$  for Adélie and  $s=2$  for emperor penguins. The probability that concentrations differed between species was determined by comparing the posterior distributions of  $\mu_s$ :  $\Pr(\text{Adélie} > \text{emperor}) = \mu_1 > \mu_2$ .

When comparing the Hg concentration between species, we included both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  as covariates. These were modelled similar to the above, allowing for different slopes for each species and random effect of year:

$$\mu_{[i]} = \beta_{[\text{spp}[i]]} + \beta_{\text{C}[\text{spp}[i]]} \times \text{C}_{[i]} + \beta_{\text{N}[\text{spp}[i]]} \times \text{N}_{[i]} + \gamma_{[\text{year}[i]]}$$

All models were fitted in the program JAGS called from R 3.3.3 (R Core Team, 2017) using the 'runjags' package (Denwood, 2016). For each model, two chains with different initial values were run for 100,000 iterations after a 10,000 iteration adaptive phase and 20,000 burn-in. All parameters were checked for 'convergence' as determined by a Gelman-Rubin statistic  $< 1.05$ .

### 3. Results

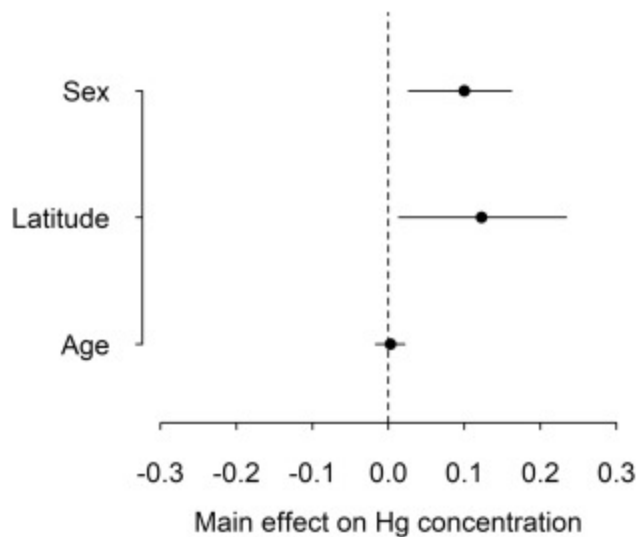
#### 3.1. Effect of sex and breeding location on Hg concentrations in Adélie penguins

Mercury concentrations were determined in Adélie penguin adults from Cape Bird ( $n=154$ ) Cape Hallett ( $n=10$ ) and Cape Adare ( $n=10$ ) between 2004 and 2016 (Table 1). The sex of 30 Adélie penguin adults from Cape Bird sampled between 2005 and 2007 was determined (Table 1). The age of 47 individuals from Cape Bird was also determined in 2009, 2010 and 2016 (Table 1).

*Observed data:* The overall measured mean concentrations of Hg in Adélie penguins was  $0.581 \mu\text{g}\cdot\text{g}^{-1}$  [SD=0.169]. Female Adélie penguins (mean  $0.617 \mu\text{g}\cdot\text{g}^{-1}$  [SD=0.126]) had lower Hg concentrations than males= $0.815 \mu\text{g}\cdot\text{g}^{-1}$  [SD=0.189]. The overall measured mean concentration of Hg was lower than both the mean concentrations for male and female Adélie penguins is due to the overall mean being across 174 birds, whereas the sex-specific means were from 30 individuals. The mean concentration of Hg in Adélie penguins breeding at higher latitudes (i.e. Cape Bird; mean= $0.592 \mu\text{g}\cdot\text{g}^{-1}$ ; SD=0.173) was higher than birds at lower latitudes (i.e. Cape Hallett and Cape Adare; mean= $0.498 \mu\text{g}\cdot\text{g}^{-1}$ ; SD=0.098).

Estimates from the linear model showed strong evidence for an effect of the sex of Adélie penguins, with a probability of 0.99 that males have a higher Hg concentration than females, with a mean difference of  $0.100 \mu\text{g}\cdot\text{g}^{-1}$  (95% CI [0.026, 0.162]; Fig. 1). Similarly, there

was strong evidence for an effect of latitude, with a probability of 0.98 that birds sampled at Cape Bird have a higher Hg concentration than those birds sampled at Cape Adare and Cape Hallett, with a mean difference of  $0.123 \mu\text{g}\cdot\text{g}^{-1}$  (95% CI [0.013, 0.233]). There was no evidence for an effect of age on the concentration of Hg in Adélie penguins ( $\text{Beta}_{\text{Age}}=0.003 \mu\text{g}\cdot\text{g}^{-1}$ , 95% CI [-0.017, 0.021]). Similarly, there was no significant change in total Hg concentrations with time in Adélie penguin feathers collected between 2004 and 2016 ( $P>.05$ ).



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Fig. 1. Beta coefficients for the three fixed effects: sex (effect of males relative to females), latitude (effect of higher latitudes relative to lower latitudes), and age of Adélie penguin adults sampled at Cape Bird, Cape Hallett and Cape Adare, in the Ross Sea, Antarctica between 2004 and 2016. For each effect, a 95% Credible Interval (as indicated by the horizontal line) that does not include 0 indicates a probability  $<.025$  that there is no effect on Hg concentration.

### 3.2. Differences in concentrations of trace metals in Adélie versus Emperor penguins

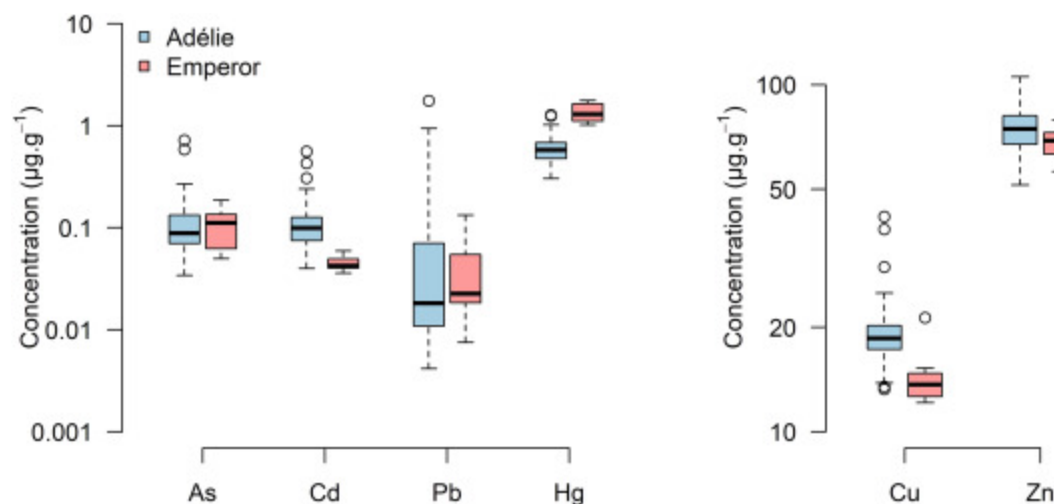
Concentrations of As, Cd, Cu, Hg, Pb and Zn were measured in 164 emperor and Adélie penguins in high latitude colonies ( $n=10$  emperor penguins at Cape Crozier, and  $n=154$  Adélie penguins at Cape Bird). Of those, 108 birds had corresponding measurements of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  ( $n=8$  emperor penguins and  $n=98$  Adélie penguins).

There was strong evidence ( $\text{Pr}(\text{Adélie}>\text{emperor})<0.01$ ) of a higher concentration of Hg in emperor penguins compared with Adélie penguins (Table 2), with the mean concentration

in emperor penguins ( $Hg_{\text{Emperor}}=1.351 \mu\text{g}\cdot\text{g}^{-1}$ ) more than twice as high than in Adélie penguins ( $Hg_{\text{Adélie}}=0.592 \mu\text{g}\cdot\text{g}^{-1}$ ; Fig. 2). In contrast, there was strong evidence ( $\text{Pr}(\text{Adélie} > \text{emperor}) > 0.98$ ) that the mean concentrations of Cd, Cu and Zn were higher in Adélie penguins compared with emperor penguins (Table 2; Fig. 2). There was no evidence of a difference in the concentrations of As nor Pb between Adélie and emperor penguins.

Table 2. Estimated mean and SD concentrations  $\mu\text{g}\cdot\text{g}^{-1}$  of trace metals in Adélie (*Pygoscelis adeliae*) and Emperor penguins (*Aptenodytes forsteri*) based on breast feather samples collected from birds breeding at high latitude colonies (Cape Bird and Cape Crozier respectively) in the Ross Sea, Antarctica, along with the probability that the mean concentration in Adélie penguins is greater than in Emperor penguins.

Trace metal	Adélie penguin		Emperor penguin		Pr(Adélie > Emperor)
	Mean	SD	Mean	SD	
As	0.111	0.006	0.105	0.025	0.59
Cd	0.111	0.005	0.044	0.020	0.99
Cu	19.00	0.28	14.32	1.01	0.99
Hg	0.592	0.015	1.351	0.058	0.00
Pb	0.092	0.016	0.046	0.062	0.76
Zn	74.74	0.79	68.20	2.94	0.98



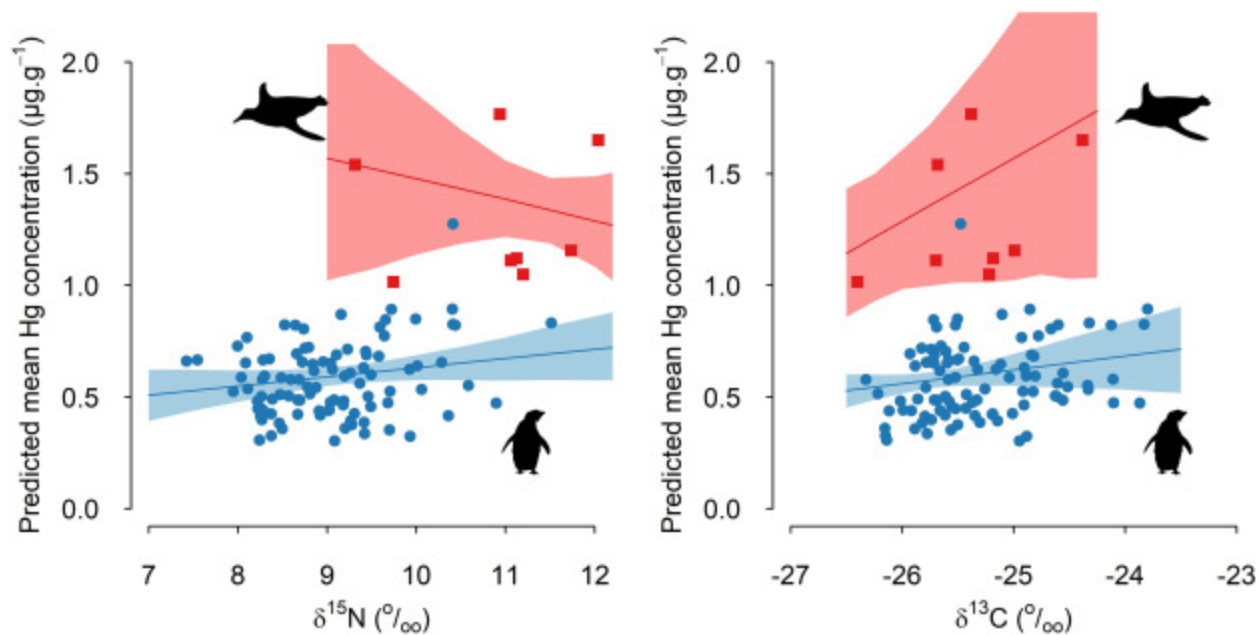
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Fig. 2. Boxplot of trace metals concentrations measured in breast feathers from 154 Adélie penguins (*Pygoscelis adeliae*) and 10 emperor penguins (*Aptenodytes forsteri*) at high latitude colonies (Cape Bird and Cape Crozier respectively) in the Ross Sea, Antarctica. Note that y-axis is on the log scale and differs between groups of elements.

### 3.3. Relationship between Hg and isotope covariates in Adélie vs emperor penguins

There was moderate evidence of a positive relationship between Hg and both  $\delta^{15}\text{N}$  ( $\text{Pr}[\beta_{\text{N}} > 0] = 0.949$ ) and  $\delta^{13}\text{C}$  ( $\text{Pr}[\beta_{\text{C}} > 0] = 0.924$ ) for Adélie penguins (Fig. 3). There was also moderate evidence of a positive relationship between Hg and  $\delta^{13}\text{C}$  for emperor penguins ( $\text{Pr}[\beta_{\text{C}} > 0] = 0.946$ ), but not for  $\delta^{15}\text{N}$  ( $\text{Pr}[\beta_{\text{N}} > 0] = 0.21$ ) (Fig. 3). The mean value for  $\delta^{15}\text{N}$  in emperor penguins (mean = 10.89, SD = 0.93) was higher than that for Adélie penguins (mean = 9.01, SD = 0.74;  $t = 5.6$ ,  $p = .0006$ ). There was no evidence for a difference in the mean value for  $\delta^{13}\text{C}$  in emperor penguins (mean = -25.36, SD = 0.59), compared with Adélie penguins (mean = -25.27, SD = 0.58;  $t = 0.44$ ,  $p = .66$ ).



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Fig. 3. Concentrations of Hg and  $\delta^{15}\text{N}$  (left) and  $\delta^{13}\text{C}$  (right) in Adélie penguins (*Pygoscelis adeliae*; blue circles) and emperor penguins (*Aptenodytes forsteri*; red squares), with the fitted relationship between Hg and the isotopes indicated by the solid line and shaded 95% Credible Intervals.

## 4. Discussion

Mercury concentrations found in Adélie and emperor penguins in our study are at the lower end of the spectrum compared with penguin species studied elsewhere in the Southern Hemisphere ([Brasso et al., 2015](#)). In a comprehensive assessment of mercury exposure in penguin populations throughout the Southern Hemisphere, the feathers of some penguin species contained up to approximately 5 µg/g of feather total Hg [e.g. gentoo penguins (*P. papua*) in the Kerguelen Islands, little penguins (*Eudyptula novaehollandiae*) in Australia and southern rockhopper penguins (*Eudyptes chrysocome*) in South America – [Brasso et al., 2015](#)]. Similarly, in a review of studies that investigated Hg in penguin feathers ([Espejo et al., 2017](#)), few detected Hg levels in the range (5–40 µg/g) linked to reduced hatching of eggs laid by different bird species ([Eisler, 1987](#)), or that can decrease reproductive success (9–20 µg/g; [Beyer et al., 1997](#); [Evers et al., 2008](#)). In accordance with the findings from our study, the review found that Hg concentrations reported in penguin feathers were generally below those known to cause adverse health and reproductive effects in birds ([Espejo et al., 2017](#)). However, it was acknowledged by [Brasso et al. \(2015\)](#) that even within penguin species there was some variability in Hg concentrations reported.

The correlation between Hg concentrations and both carbon and nitrogen stable isotopes in Adélie penguins indicates that both prey type and foraging habitat may help to explain observed Hg loadings. While no correlation was found in the current study between feather Hg concentrations and nitrogen stable isotope levels in emperor penguins, this may be the result of a small sample size. Alternatively, it may suggest that factors other than prey composition and foraging area (for example, physiology) might be influencing Hg levels in emperor penguins. Unlike albatross which have slow moult patterns and replace their plumage only every few years ([Furness, 1988](#)), both Adélie and emperor penguins under-go a complete moult annually giving both species the opportunity for detoxification of Hg. Differential moult patterns therefore, are unlikely to explain differences in Hg levels between these two species. Adélie penguins had higher concentrations of cadmium, copper and zinc than emperor penguins. Inter-specific differences in concentrations of cadmium, copper and zinc may be due to differences in diet ([Jerez et al., 2011](#)) and/or metal kinetics (the extent to which metals are absorbed, stored and eliminated ([Burger and Gochfeld, 2000](#))).

Prey type consumed influences trophic position and Hg levels in penguins.

Emperor penguins had higher total Hg concentrations and nitrogen stable isotope compositions than Adélie penguins. We attribute this finding to emperor penguins predominantly feeding on larger multi-year prey that occupy a higher trophic position than



that of the predominant prey of Adélie penguins. Adélie penguins feed extensively on krill (e.g. *Euphausia superba* and *E. crystallorophias*) but also fish such as Antarctic silverfish, *Pleuragramma antarcticum* (Coria et al., 1995; Ainley et al., 2003) or bald rockcod, *Pagothenia borchgrevinki* (Kato et al., 2003). In contrast, emperor penguins consume more Antarctic silverfish and a smaller proportion of other fish species, cephalopods, and krill (Gales et al., 1990; Cherel and Kooyman, 1998; Cherel, 2008). The biomagnification of Hg through the food chain is common for organisms that occupy a higher trophic position (Gray, 2002; Aronson et al., 2011, Cossa et al., 2011). Krill occupy a lower trophic position than Antarctic silverfish therefore a diet of krill is likely to carry an overall lower loading of Hg (Cherel, 2008; Carravieri et al., 2016). The only other known study comparing total Hg concentrations in the feathers of emperor penguins and Adélie penguins reported no difference between them but was limited by a small sample size ( $n=3$  individuals per species from Victoria Land, Ross Sea; Bargagli et al., 1998).

The size class and age of individuals within prey may also affect Hg body burden in emperor and Adélie penguins. Emperor penguins have a significantly larger body size than Adélie penguins with a corresponding larger gape and deeper diving capabilities (Chappell et al., 1993), affording greater ability to forage for a larger size class of prey items. Larger prey items tend to occupy a higher trophic position (Riede et al., 2011) and may mean greater Hg body burden to transfer to consumers. The larger size class Antarctic silverfish which emperor penguins consume may also tend to be older (Burns and Kooyman, 2001). Older prey items are expected to have bioaccumulated a greater quantity of Hg compared with younger counterparts (Mason et al., 2000), which can be passed on to the predators as they are consumed.

The carbon stable isotope values in feathers of Adélie and emperor penguins in this study were similar, suggesting that the two species are likely to be foraging in similar habitats at the time that new feathers are grown (a similar result to that reported by Carravieri et al., 2016). However, the carbon stable isotope values in both species of penguin in this study were lower than those measured in samples from Adélie Land (Carravieri et al., 2016), indicating that the individuals sampled in the Ross Sea could be favouring prey (that feed on phytoplankton) in more open sea environments, rather than prey (that feed on ice-algae at the base of the food chain) in ice-associated foraging habitats.

Inferences drawn between feather Hg concentrations and stable isotope composition may be somewhat undermined by the 'temporal mismatch' between the time of stable isotope incorporation into feathers (which only represent diet during feather growth) and the time of Hg accumulation in feathers (during feather growth and also from Hg stored in soft

tissues during inter-moult period; [Carravieri et al., 2013](#)). Stable isotope analysis is also inherently restricted to the detection of major trends ([Jarman et al., 2013](#)) and cannot provide detail about exact diet composition. Two individuals could consume different prey, but depending on the relative combinations and proportions assimilated, may have the same stable isotope signature ([Bond and Jones, 2009](#)). However, alternative assessment of Hg levels against stomach content is limited to informing on ingestion rather than assimilation is influenced by prey item durability.

#### 4.1. Sex-related differences in Hg concentrations in Adélie penguins

Male Adélie penguins from Cape Bird had a significantly higher feather mean total Hg concentration than female Adélie penguins. Differences in Hg concentrations and  $\delta^{15}\text{N}$  values between male and female Adélie penguins from Cape Bird suggest sex-related differences in prey selection and consumption. Male Adélie penguins tend to be larger and heavier than females, have a greater bill length (gape to tip) and width of bill at gape ([Ainley and Emison, 1972](#)) potentially allowing males to more effectively target different species (e.g., Antarctic silverfish) and/or larger and older individuals of the same species, than female birds. Larger prey tends to occupy a higher trophic position ([Romanuk et al., 2011](#)) and correspondingly tend to contain greater concentrations of Hg to transfer to consumers ([Atwell et al., 1998](#)). Stomach sample analysis of Adélie penguins indicated that males consumed both larger euphausiids and larger fish than female birds ([Ainley and Emison, 1972](#)). Fish generally occupy a higher trophic position than krill (e.g. Antarctic silverfish =  $10.6 \pm 0.3$   $\delta^{15}\text{N}$  (‰) versus Antarctic krill =  $5.5 \pm 0.4$   $\delta^{15}\text{N}$ , [Cherel, 2008](#)). Therefore, the higher  $\delta^{15}\text{N}$  of male Adélie penguins that breed at Cape Bird supports an assumption that they are preferentially selecting fish over krill as part of their diet. This was demonstrated in one study whereby male Adélie penguins consumed proportionally more fish during the chick-rearing phase than females, which consumed a greater proportion of krill ([Clarke et al., 1998](#)). Similarly, male gentoo penguins had higher Hg concentrations and  $\delta^{15}\text{N}$  values than females, but also Hg increased in males with increasing weight and  $\delta^{15}\text{N}$  values ([Becker et al., 2002](#); [Pedro et al., 2015](#)). These higher Hg and  $\delta^{15}\text{N}$  values have been linked to the greater physiological capacity of male gentoo penguins to dive to deeper depths and therefore access a greater range of prey items ([Bearhop et al., 2006](#); [Pedro et al., 2015](#)). Supporting these findings was a study that showed gentoo penguins that dived deeper tended to have predominantly more fish than krill in their diet ([Croxall et al., 1988](#)).

Alternatively, it is possible that female Adélie penguins have lower feather total Hg concentrations because of greater abilities to detoxify and/or excrete Hg than males. While

males are limited to excreting Hg through faeces, urine or feather moult, females may additionally deposit Hg in their eggs (Braune and Gaskin, 1987). Adélie penguin eggs reportedly contain a similar Hg concentration as the female parent bird. This suggests that egg laying is an important means by which females may excrete Hg and reduce body burden (Becker et al., 2002). The combination of physiological or anatomical dimorphism between sexes allowing male Adélie penguins to potentially forage more on fish and larger prey items, and the greater capacity of female penguins to detoxify and/or excrete Hg than males, means that male birds could be more at risk from Hg, than female birds. Other factors such as differences in metabolism, and hormonal or reproductive state between the sexes (Burger and Gochfeld, 2004) may also account for differing abilities to process and excrete Hg in the body (Monteiro and Furness, 2001). Given that all Adélie penguins that were sexed [ $n=30$ ] were from Cape Bird, it is possible that the higher Hg concentration in male Adélie penguins does not hold for other locations. It should be recognised therefore, that sexual segregation in Hg levels and trophic position in penguins and other seabirds is not always evident. Regional, temporal and/or spatial differences in foraging habitat availability and prey abundance, composition and/or distribution are likely to play an important role in mediating sex-related specialisation in diets and subsequent Hg exposure and trophic position (Furness et al., 1990; Becker et al., 2002; Tavares et al., 2013; Polito et al., 2016).

#### 4.2. Spatial variability of Adélie penguin breeding colonies to Hg sources

Higher concentrations of Hg were found in Adélie penguin feathers from birds that bred at a high latitude (southern) colony compared with those that breed at lower latitude (northern) colonies. This difference could be attributed to the Hg within volcanic emissions into the ocean. Mt. Erebus, the only active volcano on Ross Island (Kyle et al., 1990), is proximate to Cape Bird (40km) but comparatively distant to Cape Hallett (585km) and Cape Adare (700km). Mount Melbourne is the only other volcano in Victoria Land (about 350km north of Mt. Erebus) showing relatively recent activity (Lyon and Giggenschach, 1974) a few hundred years ago (Nathan and Schulte, 1967). It is possible that Adélie penguins breeding at colonies on Ross Island have a slightly higher exposure rate to Hg through prey consumed during the austral summer months, while birds are breeding, compared with birds in the northern Ross Sea. The resulting higher body burden of Hg could then be transferred to new feathers when they are grown at the end of the breeding season.

The levels of Hg and other trace metals released from local anthropogenic point sources (e.g. Antarctic research stations) entering the Antarctic marine environment need to be assessed. Mercury in sediment near the McMurdo Research Station sewage outfall was at least ten times higher than at locations away from such concentrated, regular human presence (

[Negri et al., 2006](#)). The potential for trace metals from these point sources to be transferred up through the Antarctic marine food-chain is largely unknown and requires further enquiry.

## 5. Conclusion

The current increase in anthropogenic Hg atmospheric emissions in the southern hemisphere ([Bargagli, 2008](#)) and the growing influence of climate change in Antarctica ([Stammerjohn et al., 2012](#)) has the potential to increase the long-range transportation of Hg and its cycling into the Antarctic marine food-web. Under current climate warming predictions for Antarctica, the loss of critical habitat (e.g. sea-ice) for key prey species such as krill, could reduce their abundance through poor recruitment ([Atkinson et al., 2004](#)) resulting in a higher proportion of other prey [i.e. small pelagic fish, especially myctophids; ([Pinkerton et al., 2016](#)) which occupy a higher trophic niche and potentially have greater Hg burdens than krill] in their diet. This could contribute to raising Hg levels in penguins. Added to this, declines in krill stocks could potentially increase inter-specific competition between penguins and other krill predators such as Antarctic minke whales (*Balaenoptera acutorostrata*) which have a high prevalence in waters where sea ice cover ranges between approximately 20% and 80% ([Ribic et al., 1991](#); [Ainley et al., 2012](#); [Herr et al., 2019](#)), which are also conditions preferred by Adélie penguins for foraging ([Fraser and Trivelpiece, 1996](#); [Ainley, 2002](#)). An increase in inter-specific competition for krill could push both penguin species to target a greater proportion of fish in their diets resulting in greater Hg loadings. Male Adélie penguins may be at a greater risk of accumulating higher Hg concentrations than their female counterparts because they are eating larger prey with potentially higher Hg levels and have a reduced capacity to excrete Hg than as proposed for females. This study therefore provides a baseline against which future Hg and trace metal concentrations in both emperor and Adélie penguins in the Ross Sea can be compared. As well as establishing longitudinal biomonitoring of Hg and trace metal concentrations in penguins, further studies should integrate isotopic analysis of prey in stomach contents and foraging behavior within and between species. Gaining an understanding of prey consumed Adélie and emperor penguins during the non-breeding season, and how they contribute to Hg burdens in these birds, remains a challenge due to logistical difficulties working in winter polar conditions with a species' that are highly dispersed. With some regions of Antarctica, such as the Ross Sea, experiencing an increase in the number of research stations being established, investigation into local point source Hg and trace metal emissions from stations and their logistical support infrastructure, is warranted.

## CRediT authorship contribution statement

**Natalie Pilcher:** Conceptualization, Investigation, Formal analysis, Funding acquisition, Writing - original draft. **Sally Gaw:** Conceptualization, Investigation, Writing - original draft. **Regina Eisert:** Conceptualization, Investigation, Writing - original draft. **Travis W. Horton:** Investigation, Writing - original draft. **Andrew M. Gormley:** Formal analysis, Writing - original draft. **Theresa L. Cole:** Investigation, Writing - original draft. **PPhil O'B. Lyver:** Conceptualization, Investigation, Funding acquisition, Writing - original draft.

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## Declaration of competing interest

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

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...The extent of exposure depends, among others, on diet and trophic level (Brasso et al., 2015; Espejo et al., 2017): contaminants accumulate throughout the food web, which results in apex predators showing higher concentration than their preys. For example, penguins that feed predominantly on fish generally display greater concentrations than those that feed on mollusks or crustaceans (Carravieri et al., 2013; Pilcher et al., 2020). King penguins, as long-lived top predators, are thus especially vulnerable to heavy metal pollution....

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...Notably, Adélie chicks seemed to allocate most of the Cd, Cr and Pb burden in the external tissues, potentially contributing to local contamination of terrestrial ecosystems. Alongside guano, feathers and eggs are important routes of toxic elimination by penguins (Burger, 1994; Perfetti-Bolaño et al., 2018; Pilcher et al., 2020) and contribute to the high levels of inorganic and organic contamination of the ornithogenic soils near the breeding colonies (Cipro et al., 2019; Roosens et al., 2007). With reference to the isotopic composition of eggs,  $\delta^{13}\text{C}$  values were markedly different between edible (i.e., internal organic content) and non-edible (i.e., egg membrane) parts...

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...Even so, a higher number of fish otoliths was present in female stomach samples, suggesting sexual differences in the diet of gentoo penguins (Polito et al., 2015). Based on values of  $\delta^{15}\text{N}$ , Pilcher et al. (2020) also suggested sexual intraspecific differences in prey selection and consumption, with male Adélie penguins feeding preferentially on more fish than females. A review of the use of feathers as a biomonitoring tool for organochlorine compounds showed that most studies on OCs in feathers from different bird species and locations found measurable concentrations of  $\Sigma\text{DDT}$ , HCB,  $\Sigma\text{HCH}$  and  $\Sigma\text{PCB}$  (García-Fernández et al., 2013), supporting the use of feathers in POP monitoring studies...

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