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

Natalie Pilcher^a, Sally Gaw^a, Regina Eisert^b, Travis W. Horton^c, Andrew M. Gormley^d, Theresa L. Cole^e, Phil O'B. Lyver^{d,*}

^a School of Physical and Chemical Sciences, University of Canterbury, New Zealand

^b Gateway Antarctica, Department of Geography, University of Canterbury, New Zealand

^c Department of Geology, University of Canterbury, New Zealand

^d Manaaki Whenua Landcare Research, PO Box 69040, Lincoln 7640, New Zealand

^e Department of Biology, University of Copenhagen, DK-2100 Copenhagen, Denmark

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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.

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 Nation's 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

* Corresponding author.

E-mail address: lyverp@landcareresearch.co.nz (P.O. Lyver).

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, 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 ~600 km 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 1800 km 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. 64,000 km 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 ± 0.08 kg; Cockrem et al., 2006) and emperor penguins (38.2 ± 0.7 kg; 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}N is preferentially fractionated into body tissues in comparison to ^{14}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}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}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

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 | |

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 ($-20\text{ }^{\circ}\text{C}$) in the laboratory.

2.2. Feather analysis

Each feather sample encompassed up to three feathers from a single individual. Feather samples were washed with ultrapure water ($> 18\text{ mega-ohms}$) 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 $35\text{ }^{\circ}\text{C}$ 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_3) 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 $85\text{ }^{\circ}\text{C}$ for 2 h and left to cool overnight, before 2.5 mL of 2% $\text{HNO}_3/0.5\%$ 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 $0.1\text{--}1000\text{ }\mu\text{g L}^{-1}$ 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 $1050\text{ }^{\circ}\text{C}$ under a continuous flow (c. 110 mL min^{-1}) of ultra-high-purity helium ($> 99.999\%$). The produced N_2 and CO_2 were separated using a gas chromatography column held at a constant $45\text{ }^{\circ}\text{C}$. 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 $< 0.06\text{‰}$ 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 $< 0.20\text{‰}$ 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 24 h in $40\text{ }\mu\text{L}$ Proteinase K and ATL buffer at $56\text{ }^{\circ}\text{C}$, and raising the temperature to $60\text{ }^{\circ}\text{C}$ for the final 15 min of the digest, with samples being re-suspended every 30 min at 450 RPM on a Thermomixer Comfort (Eppendorf) for 20 s. Following digestion, samples were incubated at $70\text{ }^{\circ}\text{C}$ in AL buffer for 45 min and resulting DNA was precipitated in cold 100% ethanol. DNA was eluted twice in $100\text{ }\mu\text{L}$ AE buffer, first at $70\text{ }^{\circ}\text{C}$ for 15 min, then following an initial centrifugation step the AE buffer was recycled to the spin column membrane and incubated at $70\text{ }^{\circ}\text{C}$ 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 $5\text{ }\mu\text{L}$ DNA on a 2% agarose gel electrophoresis at 75 V. Samples that resulted in a low DNA yield $< 0.50\text{ ng}/\mu\text{L}$ 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 ($10\text{ }\mu\text{L}$) were performed using 1x PCR Buffer, $250\text{ }\mu\text{M}$ each dNTP, 1.25 U i-Taq (iNTRON), $0.25\text{ }\mu\text{M}$ each primer, and $1\text{ }\mu\text{L}$ DNA extract on a BIO-RAD MyCycler thermal cycler with an initial denaturation of $94\text{ }^{\circ}\text{C}$ for 5 min, followed by 40 cycles of $94\text{ }^{\circ}\text{C}$ for 30 s, $55\text{ }^{\circ}\text{C}$ for 45 s and $72\text{ }^{\circ}\text{C}$ for 45 s, and a final 10 extension at $72\text{ }^{\circ}\text{C}$ for 10 min. Amplified products were visualised on a 3% agarose gel electrophoresis at 85 V, 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 (β_{sex}) was specified as the effect of males relative to females. The effect of latitude (β_{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 λ 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; Sex_i ~ Bernoulli(0.5) and Age_i ~ 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:

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

where

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

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 μ_s : Pr(Adélie > 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:

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

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 (Beta_{Age} = 0.003 $\mu\text{g}\cdot\text{g}^{-1}$, 95% CI [-0.017, 0.021]). Similarly, there was

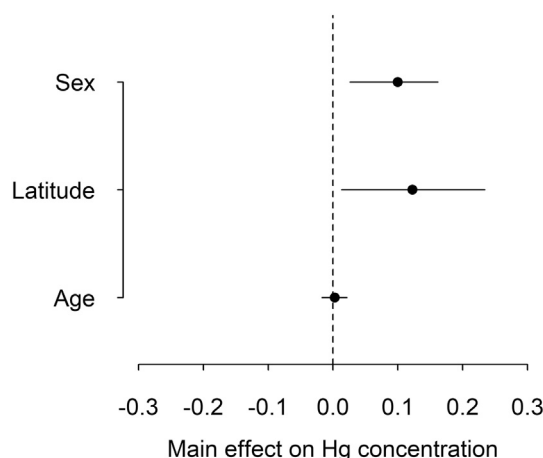


Fig. 1. Beta coefficients for the three fixed effects: sex (effect of males relative to females), latitude (effect of higher latitudes relative to lower latitudes), and age of Adélie penguin adults sampled at Cape Bird, Cape Hallett and Cape Adare, in the Ross Sea, Antarctica between 2004 and 2016. For 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.

no significant change in total Hg concentrations with time in Adélie penguin feathers collected between 2004 and 2016 (*P* > .05).

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 (Pr(Adélie > 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_{Emperor} = 1.351 $\mu\text{g}\cdot\text{g}^{-1}$) more than twice as high than in Adélie penguins (Hg_{Adélie} = 0.592 $\mu\text{g}\cdot\text{g}^{-1}$; Fig. 2). In contrast, there was strong evidence (Pr(Adélie > 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 |

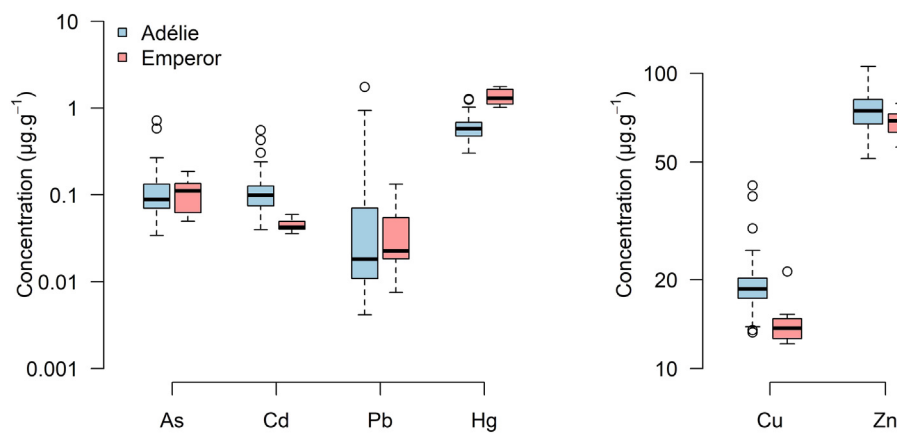


Fig. 2. Boxplot of trace metals concentrations measured in breast feathers from 154 Adélie penguins (*Pygoscelis adeliae*) and 10 emperor penguins (*Aptenodytes forsteri*) at high latitude colonies (Cape Bird and Cape Crozier respectively) in the Ross Sea, Antarctica. Note that y-axis is on the log scale 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; $t = 0.44, p = .66$).

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

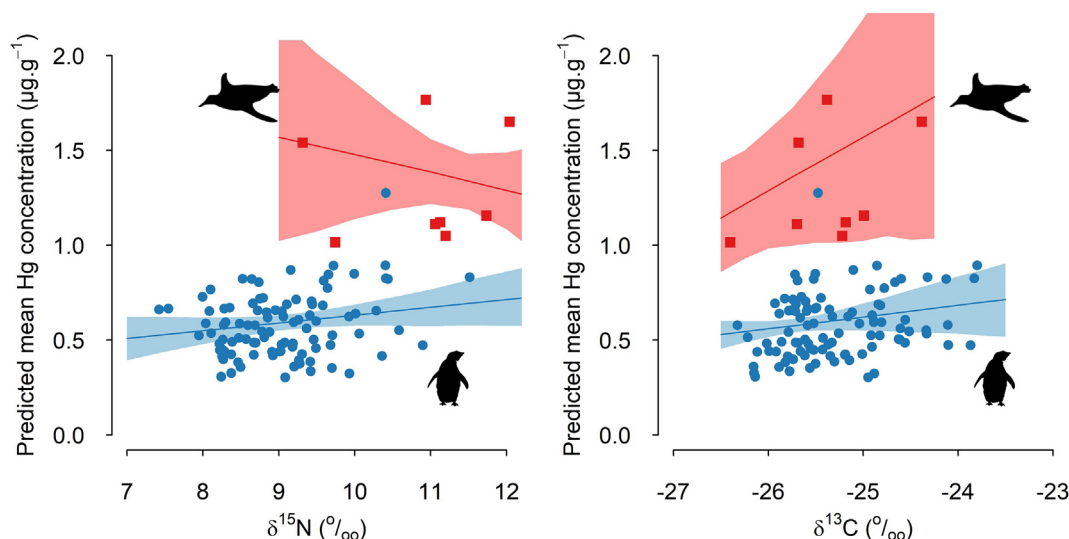


Fig. 3. Concentrations of Hg and $\delta^{15}\text{N}$ (left) and $\delta^{13}\text{C}$ (right) in Adélie penguins (*Pygoscelis adeliae*; blue circles) and emperor penguins (*Aptenodytes forsteri*; red squares), with the fitted relationship between Hg and the isotopes indicated by the solid line and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

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. crystallophias*) 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 (40 km) but comparatively distant to Cape Hallett (585 km) and Cape Adare (700 km). Mount Melbourne is the only other volcano in Victoria Land (about 350 km north of Mt. Erebus) showing relatively recent activity (Lyon and Giggenbach, 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.

References

- Ainley, D.G., 2002. The Adélie Penguin: Bellwether of Climate Change. Columbia University Press, New York, USA (416 p).
- Ainley, D.G., Emison, W.B., 1972. Sexual size dimorphism in Adélie penguins. *Ibis* 114, 267–271.
- Ainley, D.G., Ballard, G., Barton, K.J., Karl, B.J., Rau, G.H., Ribic, C.A., Wilson, P.R., 2003. Spatial and temporal variation of diet within a presumed metapopulation of Adélie penguins. *Condor* 105, 95–106.
- Ainley, D.G., Jongsomjit, D., Ballard, G., Thiele, D., Fraser, W.R., Tynan, C.T., 2012. Modeling the relationship of Antarctic minke whales to major ocean boundaries. *Polar Biol.* 35, 281–290.
- Ariya, P.A., Dastoor, A., Amyot, M., Schroeder, W., Barrie, L., Anlauf, K., Raofie, F., Ryzhkov, A., Davignon, D., Lalonde, J., Steffen, A., 2004. The Arctic: a sink for Hg. *Tellus B: Chem. Phys. Meteor.* 56, 397–403.
- Aronson, R.B., Thatje, S., McClintock, J.B., Hughes, K.A., 2011. Anthropogenic impacts on marine ecosystems in Antarctica. *Year Ecol. Con. Bio.* 1223, 82–107.
- Atkinson, A., Siegel, V., Pakhomov, E., Rothery, P., 2004. Long-term decline in krill stock and increase in salps within the Southern Ocean. *Nature* 432, 100–103.
- Atwell, L., Hobson, K.A., Welch, H.E., 1998. Biomagnification and bioaccumulation of mercury in an arctic marine food web: insights from stable nitrogen isotope analysis. *Can. J. Fish. Aquat. Sci.* 55, 1114–1121.
- Ballard, G., Toniolo, V., Ainley, D.G., Parkinson, C.L., Arrigo, K.R., Trathan, P.N., 2010. Responding to climate change: Adélie penguins confront astronomical and ocean boundaries. *Ecology* 91 (7), 2056–2069.
- Bargagli, R., 2008. Environmental contamination in Antarctic ecosystems. *Sci. Tot. Environ.* 400, 212–226.
- Bargagli, R., Monaci, F., Sanchez-Hernandez, J.C., Cateni, D., 1998. Biomagnification of mercury in an Antarctic marine coastal food web. *Mar. Ecol. Prog. Ser.* 169, 65–76.
- Bearhop, S., Phillips, R.A., Thompson, D.R., Waldron, S., Furness, R.W., 2000. Variability in mercury concentrations of great skuas *Catharacta skua*: the influence of colony, diet and trophic status inferred from stable isotope signatures. *Mar. Ecol. Prog. Ser.* 195, 261–268.
- Bearhop, S., Waldron, S., Votier, S.C., Furness, R.W., 2002. Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. *Physio. Bio. Zool.* 75 (5), 451–458.
- Bearhop, S., Richard, A., Phillips, R.A., McGill, R., Cheral, Y., Dawson, D.A., Croxall, J.P.,

2006. Stable isotopes indicate sex-specific and long-term individual foraging specialisation in diving seabirds. *Mar. Ecol. Prog. Ser.* 311, 157–164.
- Becker, P.H., González-Solís, J., Behrends, B., Croxall, J., 2002. Feather mercury levels in seabirds at South Georgia: influence of trophic position, sex and age. *Mar. Eco. Prog. Ser.* 243, 261–269.
- Beyer, N.W., Spalding, M., Morrison, D., 1997. Mercury concentrations in feathers of wading birds from Florida. *Ambio* 26, 97–100.
- Block, R.J., 1951. Chemical classification of keratins. *Ann. New York Aca. Sci.* 53, 608–612.
- Bond, A., Lavers, J.L., 2011. Trace element concentrations in feathers of flesh-footed shearwaters (*Puffinus carneipes*) from across their breeding range. *Arch. Environ. Contam. Tox.* 61 (2), 318–326.
- Bond, A.L., Jones, I.L., 2009. A practical introduction to stable-isotope analysis for seabird biologists: approaches, cautions and caveats. *Mar. Ornith.* 37, 183–188.
- Brasso, R.L., Polito, M.J., Emslie, S.D., 2014. Multi-tissue analyses reveal limited inter-annual and seasonal variation in mercury exposure in an Antarctic penguin community. *Ecotox* 23, 1494–1504.
- Brasso, R.L., Chiaradia, A., Polito, M.J., Rey, A.R., Emslie, S.D., 2015. A comprehensive assessment of mercury exposure in penguin populations throughout the Southern Hemisphere: using trophic calculations to identify sources of population-level variation. *Mar. Poll. Bull.* 97, 408–418.
- Braune, B.M., Gaskin, D.E., 1987. Mercury levels in Bonaparte's Gulls (*Larus philadelphia*) during autumn moult in the Quoddy Region, New Brunswick, Canada. *Arch. Environ. Contam. Tox.* 16, 539–549.
- Burger, J., Gochfeld, M., 2000. Metal levels in feathers of 12 species of seabirds from Midway Atoll in the northern Pacific Ocean. *Sci. Total Environ.* 257 (1), 37–52.
- Burger, J., Gochfeld, M., 2002. Effects of chemicals and pollution on seabirds. pp. 485–525; In: *Biology of Marine Birds*. E.A. Schreiber and J. Burger J, (eds). CRC Press; Boca Raton, Florida, USA.
- Burger, J., Gochfeld, M., 2004. Marine birds as sentinels of environmental pollution. *EcoHealth* 1, 263–274.
- Burns, J.M., Kooyman, G.L., 2001. Habitat use by Weddell seals and Emperor penguins foraging in the Ross Sea, Antarctica. *Amer. Zool.* 41 (1), 90–98.
- Carravieri, A., Bustamante, P., Churlaud, C., Cherel, Y., 2013. Penguins as bioindicators of mercury contamination in the Southern Ocean: birds from the Kerguelen Islands as a case study. *Sci. Tot. Environ.* 454–455, 141–148.
- Carravieri, A., Bustamante, P., Churlaud, C., Fromant, A., Cherel, Y., 2014a. Moulting patterns drive within-individual variations of stable isotopes and mercury in seabird body feathers: implications for monitoring of the marine environment. *Mar. Biol.* 161, 963–968.
- Carravieri, A., Cherel, Y., Blévin, P., Brault-Favrou, M., Chastel, O., Bustamante, P., 2014b. Mercury exposure in a large subantarctic avian community. *Environ. Poll.* 190, 51–57.
- Carravieri, A., Cherel, Y., Jaeger, A., Churlaud, C., Bustamante, P., 2016. Penguins as bioindicators of mercury contamination in the southern Indian Ocean: geographical and temporal trends. *Environ. Poll.* 213, 195–205.
- Chappell, M.A., Shoemaker, V.H., Janes, D.N., Bucher, T.L., Maloney, S.K., 1993. Diving behavior during foraging in breeding Adélie penguins. *Ecology* 74 (4), 1204–1215.
- Cherel, Y., 2008. Isotopic niches of emperor and Adélie penguins in Adélie Land, Antarctica. *Mar. Biol.* 154, 813–821.
- Cherel, Y., Kooyman, G.L., 1998. Food of emperor penguins (*Aptenodytes forsteri*) in the western Ross Sea, Antarctica. *Mar. Biol.* 130, 335–344.
- Clarke, J., Manly, B., Kerry, K., Gardner, H., Franchi, E., Corsolini, S., Focardi, S., 1998. Sex differences in Adélie penguin foraging strategies. *Pol. Biol.* 20, 248–258.
- Cockrem, J.F., Potter, M.A., Candy, E.J., 2006. Corticosterone in relation to body mass in Adélie penguins (*Pygoscelis adeliae*) affected by unusual sea ice conditions at Ross Island, Antarctica. *Gen. Comp. Endo.* 149 (3), 244–252.
- Coria, N.R., Spairani, H., Vivequin, S., Fontana, R., 1995. Diet of Adélie penguins *Pygoscelis adeliae* during the post-hatching period at Esperanza Bay, Antarctica, 1987/88. *Pol. Biol.* 15, 415–418.
- Cossa, D., Heimbürger, L.E., Lannuzel, D., Rintoul, S.R., Butler, C.V., Bowie, A.R., Avery, B., Watson, R.J., Remenyi, T., 2011. Mercury in the Southern Ocean. *Geo. et Cosmo. Acta* 75, 4037–4052.
- Croxall, J.P., Davis, R.W., O'Connell, M.J., 1988. Diving patterns in relation to diet of gentoo and macaroni penguins at South Georgia. *Condor* 90 (1), 157–167.
- Deniro, M., Epstein, S., 1981. Influence of diet on the distribution of nitrogen isotopes in animals. *Geo. et Cosmo. Acta* 45, 341–351.
- Denwood, M.J., 2016. runjags: an R Package providing interface utilities, model templates, parallel computing methods and additional distributions for MCMC models in JAGS. *J. Stat. Soft.* 71 (9), 1–25.
- Dietz, R., Born, E.W., Agger, C.T., Nielsen, C.O., 1995. Zinc, cadmium, mercury and selenium in polar bears (*Ursus maritimus*) from central east Greenland. *Pol. Biol.* 15 (3), 175–185.
- Ebinghaus, R., Kock, H.H., Temme, C., Einax, J.W., Lowe, A.G., Richter, A., Burrow, J.P., Schroeder, W.H., 2002. Antarctic springtime depletion of atmospheric mercury. *Environ. Sci. Tech.* 36, 1238–1244.
- Eisler, R., 1987. *Mercury hazards to fish, wildlife, and invertebrates: a synoptic review*. U.S. Fish and Wildlife Service, Washington DC, USA.
- Espejo, W., Celis, J.E., González-Acuña, D., Jara, S., Barra, R., 2014. Concentration of trace metals in excrements of two species of penguins from different locations of the Antarctic Peninsula. *Pol. Biol.* 37, 675–683.
- Espejo, W., Celis, J.E., González-Acuña, D., Banegas, A., Barra, R., Chiang, G., 2017. A global overview of exposure levels and biological effects of trace elements in penguins. *Reviews Environ. Contam. Toxicol.* 245, 1–64.
- Evers, D.C., Savoy, L.J., DeSorbo, C.R., Yates, D.E., Hanson, W., Taylor, K.M., Siegel, L.S., Cooley Jr., J.H., Bank, M.S., Major, A., Munney, K., Mower, B.F., Vogel, H.S., Schoch, N., Pokras, M., Goodale, M.W., Fair, J., 2008. Adverse effects from environmental mercury loads on breeding common loons. *Ecotoxicology* 17, 69–81.
- Fraser, W.R., Trivelpiece, W.Z., 1996. Factors controlling the distribution of seabirds: winter-summer heterogeneity in the distribution of Adélie penguin populations. *Antarct. Res. Series.* 70, 257–272.
- Fretwell, P.T., LaRue, M.A., Morin, P., Kooyman, G.L., Wienecke, B., Ratcliffe, N., Fox, A.J., Fleming, A.H., Porter, C., Trathan, P.N., 2012. An emperor penguin population estimate: the first global, synoptic survey of a species from space. *PLoS One* 7 (4), e33751.
- Furness, R.W., 1988. Influences of status and recent breeding experience on the moult strategy of the yellow-nosed albatross *Diomedea chlororhynchus*. *J. Zool. (Lond.)* 215, 719–727.
- Furness, R.W., Lewis, S.A., Mills, J.A., 1990. Mercury levels in the plumage of red-billed gulls *Larus novaehollandiae scopulinus* of known sex and age. *Environ. Poll.* 63, 33–39.
- Furness, R.W., Thompson, D.R., Becker, P.H., 1995. Spatial and temporal variation in mercury contamination of seabirds in the North Sea. *Helgo. Meeresunter.* 49, 605–615.
- Gales, N.J., Klages, N.T.W., Williams, R., Woehler, E.J., 1990. The diet of the emperor penguin, *Aptenodytes forsteri*, in Amanda Bay, Princess Elizabeth Land, Antarctica. *Ant. Sci.* 2 (1), 23–28.
- Goutte, A., Barbraud, C., Meillère, A., Carravieri, A., Bustamante, P., Labadie, P., Budzinski, H., Delord, K., Cherel, Y., Weimerskirch, H., Chastel, O., 2014a. Demographic consequences of heavy metals and persistent organic pollutants in a vulnerable long-lived bird, the wandering albatross. *Proc. Roy. Soc. B-Biol. Sci.* 281, 1787.
- Goutte, A., Bustamante, P., Barbraud, C., Delord, K., Weimerskirch, H., Chastel, O., 2014b. Demographic responses to mercury exposure in two closely related Antarctic top predators. *Ecology* 95 (4), 1075–1086.
- Gray, J.S., 2002. Biomagnification in marine systems: the perspective of an ecologist. *Mar. Poll. Bull.* 45, 46–52.
- Groscolas, R., 1986. Changes in body mass, body temperature and plasma fuel levels during the natural breeding fast in male and female emperor penguins *Aptenodytes forsteri*. *J. Comp. Physio. B-Biochem. Sys. Environ. Phys.* 156 (4), 521–527.
- Harrington, C.F., Merson, S.A., D'Silva, T.M., 2004. Method to reduce the memory effect of mercury in the analysis of fish tissue using inductively coupled plasma mass spectrometry. *Analy. Chim. Acta* 505 (2), 247–254.
- Herr, H., Kelly, N., Dorschel, B., Huntemann, M., Kock, K.-H., Lehnert, L.S., Siebert, U., Viquerat, S., Williams, R., Scheidat, M., 2019. Aerial surveys for Antarctic minke whales (*Balaenoptera bonaerensis*) reveal sea ice dependent distribution patterns. *Ecol. Evol.* 1–19.
- Hobson, K.A., Piatt, J.F., Pitocchelli, J., 1994. Using stable isotopes to determine seabird trophic relationships. *J. Ani. Ecol.* 63 (4), 786–798.
- Hobson, K.A., Ambrose Jr., W.G., Renaud, P.E., 1995. Sources of primary production, benthic-pelagic coupling, and trophic relationships within the northeast water polynya: insights from $\delta^{13}C$ and $\delta^{15}N$ analysis. *Mar. Ecol. Prog. Ser.* 128, 1–10.
- Jarman, S.N., McInnes, J.C., Faux, C., Polanowski, A.M., Marthick, J., Deagle, B.E., Southwell, C., Emmerson, L., 2013. Adélie penguin population diet monitoring by analysis of food DNA in scats. *PLoS One* 8 (12), e82227.
- Jerez, S., Motas, M., Palacios, M.J., Valera, F., Cuervo, J.J., Barbosa, A., 2011. Concentration of trace elements in feathers of three Antarctic penguins: geographical and interspecific differences. *Environ. Poll.* 159, 2412–2419.
- Kato, A., Watanuki, Y., Naito, Y., 2003. Annual and seasonal changes in foraging site and diving behavior in Adélie penguins. *Pol. Biol.* 26, 389–395.
- Kelly, J.F., 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Can. J. Zool.* 78, 1–27.
- Kessler, R., 2013. The Minamata Convention on Mercury: a first step toward protecting future generations. *Environ. Health Pers.* 121 (10), A304–A309.
- Kruschke, J.K., 2013. Bayesian estimation supersedes the t-test. *J. Exp. Psych: General.* 143, 573–603.
- Kyle, P.R., Meeker, P.R., Finnegan, D., 1990. Emission rate of sulfur-dioxide, trace gases and metals from mount Erubus, Antarctica. *Geophys. Res. Lett.* 17 (12), 2125–2128.
- Labbe, A.M.T., Dunlop, J.N., Loneragan, N.R., 2013. Central place foraging and feather regrowth rate in bridled terns (*Onychoprion anaethetus*): an insight from stable isotopes. *Mar. Freshw. Res.* 64, 1184–1191.
- Lamborg, C.H., Hammerschmidt, C.R., Bowman, K.L., Swarr, G.J., Muson, K.M., Ohnemus, D.C., Lam, P.J., Heimbürger, L.-E., Rijkenberg, M.J.A., Saito, M., 2014. A global ocean inventory of anthropogenic mercury based on water column measurements. *Nature* 512, 65–68.
- Larson, H.J., 2014. The Minamata Convention on Mercury: risk in perspective. *Lancet* 383, 198–199.
- Li, Y.F., Chen, C.Y., Li, B., Sun, J., Wang, J.X., Gao, Y.X., Zhao, Y.L., Chai, Z.F., 2006. Elimination efficiency of different reagents for the memory effect of memory using ICP-MS. *J. Anal. Atomic Spectro.* 21 (1), 94–96.
- Lock, J.W., Thompson, D.R., Furness, R.W., Bartle, J.A., 1992. Metal concentrations in seabirds of the New Zealand region. *Environ. Poll.* 75, 289–300.
- Lyon, G.L., Giggenschbach, W.F., 1974. Geothermal activity in Victoria Land, Antarctica. *N.Z. J. Geol. Geophys.* 17, 511–521.
- Lyver, P.O'B., Barron, M., Barton, K.J., Ainley, D.G., Pollard, A., Gordon, S., McNeill, S., Ballard, G., Wilson, P.R., 2014. Trends in the breeding population of Adélie penguins in the Ross Sea, 1981–2012: a coincidence of climate and resource extraction effects. *PLoS One* 9 (3), e91188.
- Lyver, P.O'B., Aldridge, S.P., Gormley, A.M., Gaw, S., Webb, S., Buxton, R.T., Jones, C.J., 2017. Elevated Hg concentrations in the feathers of grey-faced petrels (*Pterodroma gouldi*) in New Zealand. *Mar. Poll. Bull.* 119 (1), 195–203.
- Mason, R.P., Laporte, J.M., Andres, S., 2000. Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater

- invertebrates and fish. Arch. Environ. Cont. Tox. 38 (3), 283–297.
- Minagawa, M., Wada, E., 1984. Stepwise enrichment of ^{15}N along food-chains – further evidence and the relation between $\delta^{15}\text{N}$ and animal age. Geo. et Cos. Acta 48 (5), 1135–1140.
- Monteiro, L.R., Furness, R.W., 1995. Seabirds as monitors of mercury in the marine environment. Water Air Soil Poll 80 (1–4), 851–870.
- Monteiro, L.R., Furness, R.W., 2001. Kinetics, dose-response, and excretion of methylmercury in free-living adult Cory's shearwaters. Environ. Sci. Tech. 35, 739–746.
- Morel, F.M.M., Kraepiel, A.M.L., Amyot, M., 1998. The chemical cycle and bioaccumulation of Hg. Ann. Rev. Ecol. Sys. 29, 543–566.
- Muir, D.C.G., Wagemann, R., Hargrave, B.T., Thomas, D.J., Peakall, D.B., Norstrom, R.J., 1992. Arctic marine ecosystem contamination. Sci. Tot. Environ. 122, 75–134.
- Nathan, S., Schulte, F.J., 1967. Recent thermal and volcanic activity on Mount Melbourne, northern Victoria Land, Antarctica. N.Z. J. Geol. Geophys. 10, 422–430.
- Negri, A., Burns, K., Boyle, S., Brinkman, D., Webster, N., 2006. Contamination of sediments, bivalves and sponges of McMurdo Sound, Antarctica. Environ. Poll. 143, 456–467.
- Newman, M.C., 2015. Fundamentals of Ecotoxicology: The Science of Pollution. CRC Press, Boca Raton, FL, USA (680 p).
- O'Driscoll, N.J., Rencz, A., Lean, D.R.S., 2005. The biogeochemistry and fate of mercury in the environment – chapter 9. In: Sigel, A., Sigel, H., Sigel, R. (Eds.), Metal Ions in Biological Systems. Biogeochemical Cycles of Elements 43. Taylor and Francis, Boca Raton, USA, pp. 221–238.
- Park, T., Casella, G., 2008. The Bayesian Lasso. J. Amer. Stat. Assoc. 103, 681–686.
- Pedro, S., Xavier, J.C., Tavares, S., Trathan, P.N., Ratcliffe, N., Paiva, V.H., Medeiros, R., Vieira, R.P., Ceia, F.R., Pereira, E., Pardal, M.A., 2015. Hg accumulation in gentoo penguins *Pygoscelis papua*: spatial, temporal and sexual intraspecific variations. Pol. Biol. 38, 1335–1343.
- Pfaffhuber, K.A., Berg, T., Hirdman, D., Stohl, A., 2012. Atmospheric mercury observations from Antarctica: seasonal variation and source and sink region calculations. Atmos. Chem. Phys. 12, 3241–3251.
- Pinkerton, M.H., Lyver, P.O.B., Stevens, D.W., Forman, J., Eisert, R., Mormede, S., 2016. Increases in Adélie penguins in the Ross Sea: could the fishery for Antarctic toothfish be responsible? Ecol. Model. 337, 262–271.
- Pirrone, N., Hedgecok, I.M., Cinnirella, S., Sprovieri, F., 2010. Overview of major processes and mechanisms affecting the mercury cycle on different spatial and temporal scales. EPJ Web of Conferences 9, 3–33.
- Polito, M.J., Brasso, R.L., Trivelpiece, W.Z., Karnovsky, N., Patterson, W.P., Emslie, S.D., 2016. Differing foraging strategies influence Hg (Hg) exposure in an Antarctic penguin community. Environ. Poll. 218, 196–206.
- Post, D., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83 (3), 703–718.
- R Core Team, 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Ribic, C.A., Fraser, W.R., Ainley, D.G., 1991. Habitat selection by marine mammals in the marginal ice zone. Antarct. Sci. 3, 181–186.
- Riede, J.O., Brose, U., Ebenman, B., Jacob, U., Thompson, R., Townsend, C., Jonsson, T., 2011. Stepping in Elton's footprints: a general scaling model for body masses and trophic levels across ecosystems. Ecol. Lett. 14, 169–178.
- Romanuk, T.N., Hayward, A., Hutchings, J.A., 2011. Trophic level scales positively with body size in fishes. Global Ecol. Biogeog. 20, 231–240.
- Schroeder, W.H., Anlauf, K.G., Barrie, L.A., Lu, J.Y., Steffen, A., Schneeberger, D.R., Berg, T., 1998. Arctic springtime depletion of mercury. Nature 394 (6691), 331–332.
- Shaffer, S.A., Tremblay, Y., Weimerskirch, H., Scott, D., Thompson, D.R., Sagar, P.M., Moller, H., Taylor, G.A., Foley, D.G., Barbara, A., Block, B.A., Costa, D.P., 2006. Migratory shearwaters integrate oceanic resources across the Pacific Ocean in an endless summer. PNAS 103 (34), 12799–12802.
- Slemr, F., Langer, E., 1992. Increase in global atmospheric concentrations of mercury inferred from measurements over the Atlantic Ocean. Nature 344 (6359), 434–437.
- Søreide, J.E., Hop, H., Carroll, M.L., Falk-Petersen, S., Hegseth, E.N., 2006. Seasonal food web structures and sympagic-pelagic coupling in the European Arctic revealed by stable isotopes and a two-source food web model. Prog. Ocean. 71, 59–87.
- Spalding, M.G., Frederick, P.C., McGill, H.C., Bouton, S.N., McDowell, L.R., 2000. Methylmercury accumulation in tissues and its effects on growth and appetite in captive great egrets. J. Wild. Disease. 36 (3), 411–422.
- Sprovieri, F., Pirrone, N., Ebinghaus, R., Kock, H., Dommergue, A., 2010. A review of worldwide atmospheric mercury measurements. Atmos. Chem. Phys. 10 (17), 8245–8265.
- Stammerjohn, S., Massom, R., Rind, D., Martinson, D., 2012. Regions of rapid sea ice change: an inter-hemispheric seasonal comparison. Geophys. Res. Lett. 39.
- Streets, D.G., Zhang, Q., Wu, Y., 2009. Projections of global mercury emissions in 2050. Environ. Sci. Tech. 43, 2983–2988.
- Tartu, S., Goutte, A., Bustamante, P., Bustamante, Angelier, F., Moe, B., Clément-Chastel, C., Bech, C., Gabrielsen, G.W., Bustnes, J.O., Chastel, O., 2013. To breed or not to breed: endocrine response to mercury contamination by an Arctic seabird. Biol. Lett. 9 (4), 1–4.
- Tavares, S., Xavier, J.C., Phillips, R.A., Pereira, M.E., Pardal, M.A., 2013. Influence of age, sex and breeding status on mercury accumulation patterns in the wandering albatross *Diomedea exulans*. Environ. Poll. 181, 315–320.
- Wienecke, B., Kirkwood, R., Robertson, G., 2004. Pre-moult foraging trips and moult locations of Emperor penguins at the Mawson Coast. Pol. Biol. 27 (2), 83–91.
- Wolfe, M.F., Schwarzbach, S., Sulaiman, R.A., 1998. Effects of Hg on wildlife: a comprehensive review. Environ. Tox. Chem. 17 (2), 146–160.
- Zhang, P., Han, J., Liu, Q., Zhang, J., Zhang, X., 2013. Sex identification of four penguin species using locus-specific PCR. Zoo Biol 32 (3), 257–261.
- Zimmer, I., Piatkowski, U., Brey, T., 2007. The trophic link between squid and the emperor penguin *Aptenodytes forsteri* at Pointe Géologie, Antarctica. Mar. Bio. 152, 1187–1195.