Penguins as bioindicators of mercury contamination in the southern Indian Ocean: geographical and temporal trends

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Abstract
Penguins have been recently identified as useful bioindicators of mercury (Hg) transfer to food webs in the Southern Ocean over different spatial and temporal scales. Here, feather Hg concentrations were measured in adults and chicks of all the seven penguin species breeding in the southern Indian Ocean, over a large latitudinal gradient spanning Antarctic, subantarctic and subtropical sites. Hg was also measured in feathers of museum specimens of penguins collected at the same sites in the 1950s and 1970s. Our aim was to evaluate geographical and historical variations in Hg transfer to penguins, while accounting for feeding habits by using the stable isotope technique (δ13C, habitat; δ15N, diet/trophic level). Adult feather Hg concentrations in contemporary individuals ranged from 0.7 ± 0.2 to 5.9 ± 1.9 µg g⁻¹ dw in Adélie and gentoo penguins, respectively. Inter-specific differences in Hg accumulation were strong among both adults and chicks, and mainly linked to feeding habits. Overall, penguin species that feed in Antarctic waters had lower feather Hg concentrations than those that feed in subantarctic and subtropical waters, irrespective of age class and dietary group, suggesting different Hg incorporation into food webs depending on the water mass. While accounting for feeding habits, we detected different temporal variations in feather Hg concentrations depending on species. Notably, the subantarctic gentoo and macaroni penguins had higher Hg burdens in the contemporary rather than in the historical sample, despite similar or lower trophic levels, respectively. Whereas increases in Hg deposition have been recently documented in the Southern Hemisphere, future monitoring is highly needed to confirm or not this temporal trend in penguins, especially in the context of actual changing Hg emission patterns and global warming.

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1. Introduction
Mercury (Hg) is a highly-toxic nonessential metal of global concern. Under its inorganic form, Hg is highly volatile, and can be transported over inter-continental distances (UNEP, 2013). Atmospheric deposition is the main input of Hg to the open ocean (e.g., Mason et al., 2012; Driscoll et al., 2013). Hence, Hg is widely distributed in oceanic waters, with the concentrations of the different chemical species varying horizontally and vertically depending on a multitude of biotic and abiotic factors (Fitzgerald et al., 2007). Under the organic form (methyl-Hg, Me₃-Hg), Hg bioaccumulates in the tissues of marine organisms and bio-magnifies up food webs (Fitzgerald et al., 2007; Driscoll et al., 2013), exerting deleterious effects such as endocrine disruption and neurodevelopmental impairment in apex predators (e.g., Wolfe et al., 1998; Dietz et al., 2013; Tartu et al., 2013). Anthropogenic perturbations to the global Hg cycle have tripled the total Hg content of surface oceanic waters compared to pre-anthropogenic conditions, and also significantly increased total Hg concentration in intermediate and deep waters (Driscoll et al., 2013; Lamborg et al., 2014). In the Northern Hemisphere, growing oceanic Hg concentrations have been associated to increasing Hg bio-accumulation in marine biota, such as marine mammals and
seabirds (Monteiro and Furness, 1997, Braune et al., 2005; Bond et al., 2015). Comparatively less information is available on Hg concentrations and transfer to food webs in oceanic waters of the Southern Hemisphere (but see Thompson et al., 1993; Frias et al., 2012; Brasso et al., 2015). This is particularly true for the southern Indian Ocean, where studies on bioaccumulation in biota are increasing (e.g., Bocher et al., 2003; Carravieri et al., 2014a; Fontaine et al., 2015), while, surprisingly, there is still limited information on seawater Hg distribution and speciation (Cossa et al., 2011; Lamborg et al., 2014), and no data on temporal trends. Seabirds have been used extensively as bioindicators of Hg contamination of their food webs (Furness and Camphuysen, 1997). Like other apex predators, seabirds are mainly exposed to Hg through food ingestion, and feeding ecology has been shown to be the main driver of between- and within-species variations of Hg concentration in their tissues (Becker et al., 2002; Bearhop et al., 2000; Carravieri et al., 2014a, b). After absorption by the gut, Hg contained in prey is highly assimilated in internal tissues, mainly the liver, kidney and muscle (Walker et al., 2012), and it is efficiently excreted through the moult over a regular basis (Furness et al., 1986; Monteiro and Furness, 2001). Feather Hg concentrations constitute thus a pertinent proxy of the Hg body burden (Fontaine et al., 2015), while, surprisingly, there is still limited information on the temporal variations in this remote oceanic region. Recent studies of our group using seabirds suggest that Hg food web transfer to predators has a latitudinal trend in the southern Indian Ocean, increasing from Antarctic, through subtropical, to subantarctic waters (Cossa et al., 2011; Lamborg et al., 2014). Moreover, only one previous study has evaluated historical variations in Hg concentrations in a single seabird species in the southern Indian Ocean (Scheifler et al., 2005), and there is urgent need to investigate long-term temporal trends in this remote oceanic region.

Penguins represent the greatest seabird biomass in the Southern Ocean (Williams, 1995) and are particularly pertinent as bioindicator species of Hg food web contamination. Indeed, penguins are less dispersive than flying seabird species, and some populations from the southern Indian Ocean have almost constant trophic niches over the short- (breeding period) to long-term (wintering) (e.g., Thiébot et al., 2011a, b; 2012). Furthermore, penguins moult annually their entire plumage over a short-period, while fasting ashore (Williams, 1995; Cherel et al., 1994), reducing within-individual variations of Hg concentrations in their feathers (Brasso et al., 2013; Carravieri et al., 2014c). By using penguins as bioindicators, and as a companion investigation to Carravieri et al. (2013), the main aim of this study was to describe spatio-temporal variations in Hg transfer to these avian predators at different latitudes of the southern Indian Ocean. To this end, feather Hg has been measured in all penguin species breeding in the French Austral Territories (TAAF) over a large geographical range, from Antarctica to the subtropics, during the same breeding season in 2006–2007 (Table 1). Moreover, feathers were obtained from museum specimens of penguins collected at the same sites during the 1950s to the 1970s. Potential spatio-temporal variations in feeding ecology have been taken into account using stable isotopes of carbon (13C) and nitrogen (15N), which are known proxies of feeding habitat and trophic position, respectively (Vanderklift and Ponsard, 2003; Newsome et al., 2007). Hg and stable isotopes have been measured in feathers from both adults and chicks at each site. Chicks were expected to show lower feather Hg concentrations than adults, because of their shorter period of exposure. Given the previous results on the latitudinal differences in Hg transfer to predators in the southern Indian Ocean (Blevin et al., 2013; Carravieri et al., 2014b; Goutte et al., 2014), we expected increasing Hg concentrations in the feathers of penguin populations breeding from high to low latitudes. Finally, given the increasing trends of Hg deposition in the Southern Hemisphere (e.g., Hermanns and Biester, 2013) and of Hg bioaccumulation in marine animals (e.g., Vo et al., 2011; Bond et al., 2015), we expected feather Hg concentrations of contemporary penguins to be higher than those of museum specimens from the 50s and 70s.

2. Material and methods

2.1. Study site, species and field collections

Fieldwork was conducted at three sites of the French Southern and Antarctic Territories, that are representative of different water masses, namely Adélie land (66°40'S, 140°01'E) in Antarctica, Southern Ocean, the Crozet Islands (46°26'S, 51°45'E) in the subantarctic zone sensus lato (between the Polar and the Subtropical Fronts, Southern Ocean), and Amsterdam Island (37°50'S, 77°31'E) in the subtropics (north of the Subtropical Front, Indian Ocean). We define the Southern Ocean as the water masses south of the Subtropical Front (Pollard et al., 2002). For simplicity, we use the term “southern Indian Ocean” when referring to the whole region studied. All the seven penguin species breeding at these sites were sampled: emperor A. forsteri and Adélie Pygoscelis adeliae penguins at Adélie land; king A. patagonicus, macaroni E. chrysolophus, southern rockhopper E. chrysocome filholi and gentoo penguins Pygoscelis papua at Crozet Islands; and northern rockhopper penguins E. moseleyi at Amsterdam Island.

Penguin moult involves two distinct processes, with new feather synthesis and old feather loss overlapping in mid-moult (Cherel et al., 1994). Thus, for the contemporary sample, both new and old feathers from the same individual adult penguins were simultaneously collected in mid-moult, in order to evaluate potential inter-annual variation of adult penguins Hg exposure at the individual scale. Old and new feathers refer to mouls that occurred during the 2005–2006 and 2006–2007 austral summers (hereafter called 2006 and 2007), respectively. Emperor penguin feathers were collected on breeding, not moulting birds, because, unlike other penguins, adult emperor penguins do not moult at the breeding sites. Inter-annual variations in feather Hg content were thus evaluated in six species only. Feathers (2007 moult) were also sampled from chicks at fledging (i.e. at the end of the breeding season). Historical feather samples (from 1950 to 1977) were obtained from specimens held in the ornithological collection of the Muséum National d'Histoire Naturelle of Paris (France). Feathers were taken only when complete capture information was present with a particular study specimen. Between 6 and 10 body feathers per individual (or museum specimen) were collected and stored dry in sealed plastic bags until analysis at the University of La Rochelle, France.

2.2. Sample analyses

Prior to chemical analysis, feathers were cleaned to remove surface lipids and contaminants as previously described (Carravieri et al., 2013). Since almost all Hg is under the organic form in feathers, total Hg approximates the amount of feather Me–Hg (Bond and Diamond, 2009; Thompson and Furness, 1989). Total Hg was also quantified in feathers of museum specimens, because Hg-containing preservatives were not used at the Muséum National d'Histoire Naturelle of Paris at the time the museum specimens were collected, avoiding a potential contamination (Vo et al., 2011).
Following recommendations in Carravieri et al. (2014c), only one individual feather per penguin was analysed for total Hg in an Advanced Mercury Analyzer spectrophotometer (Altec AMA 254). Analyses were realised in duplicate or triplicate until having a relative standard deviation < 10%. A certified reference material (Tort-2 Lobster Hepatopancreas, NRC, Canada; certified Hg concentration: 0.27 ± 0.06 μg g⁻¹ dry mass) was used to check accuracy. Our measured values were 0.23 ± 0.003 μg g⁻¹ dry mass, n = 7. Blanks were analysed before each set of samples. The detection limit of the method was 0.005 μg g⁻¹ dry mass. Feather Hg concentrations are presented relative to the dry weight (dw).

After the cleaning procedure, an individual feather per penguin was homogenized by cutting it with scissors into small fragments, weighed (~0.3 mg) with a microbalance and packed into tin containers. The relative abundance of carbon and nitrogen isotopes were determined with a continuous flow mass spectrometer (Thermo Scientific Delta V Advantage) coupled to an elemental analyser (Thermo Scientific Flash EA 1112). Isotopic values are presented in the usual δ notation relative to Vienna Pee Dee Belemnite and atmospheric N₂ for δ¹³C and δ¹⁵N, respectively. Replicate measurements of internal laboratory standards (acetanilide) indicated measurement errors < 0.15% for both δ¹³C and δ¹⁵N values. Results of δ¹³C and δ¹⁵N values of adult penguins, but not chicks, were previously used to investigate historical changes in the isotopic niche of penguins (Jaeger and Cherel, 2011). In the present study, the isotopic data have been exploited again in order to evaluate geographical and temporal trends in Hg bioaccumulation in penguins while accounting for potential changes in their feeding ecology. In long-term analyses using contemporary and historical samples, the raw δ¹³C values of adult penguin feathers were adjusted following Hilton et al. (2006) and Jaeger and Cherel (2011) by taking into account the two effects resulting from human fossil fuel burning: first, the Suess effect (fossil carbon introduced into the atmosphere has a lower δ¹³C than background carbon, Keeling, 1979) and second, the effect of the increase in atmospheric CO₂ and thus aqueous CO₂, which increases phytoplankton fractionation, reducing its δ¹³C values (Rau et al., 1992).

### 2.3. Statistical analyses

All statistical analyses were performed using R 2.15.1 (R Core Team, 2012). Data were first checked for normality and homogeneity of variances by means of Shapiro–Wilk and Bartlett tests, respectively. Depending on the results, parametric or non-parametric tests were used. In order to test differences between contemporary and historical feather Hg, δ¹³C and δ¹⁵N values a categorical variable (epoch) was used rather than a continuous time-series, because of the small sample size and of the variability of the date of collection of museum specimens depending on species. Multifactorial analyses were realised in order to identify the most influential variables in describing geographical and temporal variation in feather Hg concentrations. The models were restricted to primary effects (explanatory variables: species, δ¹³C, δ¹⁵N and/or epoch) and two-way interactions. Feather δ¹³C and δ¹⁵N values were fitted in the same models since no collinearity was detected (all variance inflation factors < 2, Zuur et al., 2010). Forward selection using the Akaike’s Information Criterion corrected for small sample sizes (AICc) (Burnham and Anderson, 2002) was applied. Since our aim was to make inference on the variables affecting contaminant burdens, the effect of variables was inferred through Akaike’s weights (Burnham and Anderson, 2002). Model fit was checked by residual analysis (Zuur et al., 2009). A significance level of p < 0.05 was used for all tests, both in unifactorial and multifactorial analysis. Values are means ± SD.

### 3. Results

#### 3.1. Latitudinal variation in adult and chick feather Hg concentrations and influence of species and feeding habits

Feather Hg and δ¹³C and δ¹⁵N values were measured in 170 individual penguins, including adults and chicks, during the 2006–2007 austral summer season. Among adult penguins, there was a 9-fold difference in Hg concentrations between the least and the most contaminated species, namely the Adélie and gentoo penguins, respectively (Table 2). Based on 2007 feathers, inter-specific differences were strong and significant (Kruskal–Wallis, H = 67, p < 0.0001, Table 2), with Antarctic species having lower feather Hg concentrations than subantarctic and sub-Antarctic species, irrespective of the dietary group (mainly fish- or mainly crustacean-feeders, Fig. 1). Feather δ¹³C and δ¹⁵N values differed widely among adult penguins (ANOVA, F₆,₂₉₉ = 399 and 68,
respectively, both \( p < 0.0001 \), with each species having a unique isotopic niche (Table 2). Multifactorial analyses showed that the species and the \(^{15}N\) signature had the greatest explanatory power of adult feather Hg concentrations at the different sites, while \(^{13}C\) signature had a secondary role (Table 3).

Chicks had systematically lower feather Hg concentrations than adults, irrespective of species, dietary group or site (Wilcoxon comparisons, 100 < \( W < 170 \), all \( p < 0.0001 \)). Inter-specific differences in feather Hg concentrations in chicks were significant (Kruskal–Wallis, \( H = 72 \), \( p < 0.0001 \)). Among chicks, the seven species ranked in a relatively different order than adults (Table 2), but still Antarctic species had lower feather Hg concentrations than subantarctic and subtropical species. Isotopic niches were different depending on species (ANOVA, \( F_{6,76} = 691 \) and 103 for \(^{13}C\) and \(^{15}N\) values, respectively, both \( p < 0.0001 \)), with the exception of macaroni and gentoo penguin chicks at Crozet Islands, which had similar feather \(^{13}C\) and \(^{15}N\) values (Table 2). Multifactorial analyses revealed that, in contrast to adults, the \(^{13}C\) signature was as important as the species in explaining variation of chick feather Hg concentrations (Table 3).

### Table 2

Feather Hg concentrations (\( \mu g \cdot g^{-1} \cdot dw \)) and \(^{13}C\) and \(^{15}N\) values (%) of seven penguin species in 2006 and 2007. Values are means ± SD with ranges in parentheses for Hg.

<table>
<thead>
<tr>
<th>Species</th>
<th>Age class</th>
<th>Year</th>
<th>Number</th>
<th>Hg ± SD</th>
<th>Adult:Chick Hg ratio</th>
<th>(^{13}C) ± d</th>
<th>(^{15}N) ± c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adélie penguin, <em>Aptenodytes forsteri</em></td>
<td>Adult</td>
<td>Old</td>
<td>2007</td>
<td>17</td>
<td>1.77 ± 0.37 (1.05–2.76)</td>
<td>2.9</td>
<td>-23.1 ± 0.3A</td>
</tr>
<tr>
<td></td>
<td>Chick</td>
<td>New</td>
<td>2007</td>
<td>12</td>
<td>0.61 ± 0.11 (0.44–0.86)</td>
<td>-24.2 ± 0.3a</td>
<td>12.4 ± 0.4a</td>
</tr>
<tr>
<td>Crozet Islands (Subantarctic Zone)</td>
<td>Adult</td>
<td>New</td>
<td>2007</td>
<td>10</td>
<td>0.66 ± 0.20 (0.41–1.06)</td>
<td>3.5</td>
<td>-23.4 ± 0.4A</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>Old</td>
<td>2006</td>
<td>12</td>
<td>0.43 ± 0.13 (0.24–0.72)</td>
<td>-23.5 ± 0.2b</td>
<td>10.7 ± 0.4b</td>
</tr>
<tr>
<td>King penguin, <em>Aptenodytes patagonicus</em></td>
<td>Adult</td>
<td>New</td>
<td>2007</td>
<td>12</td>
<td>2.89 ± 0.73 (2.13–4.47)</td>
<td>1.6</td>
<td>-20.8 ± 0.6B</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>Old</td>
<td>2006</td>
<td>12</td>
<td>2.94 ± 0.47 (2.15–3.63)</td>
<td>-21.6 ± 0.3c</td>
<td>10.5 ± 0.4b</td>
</tr>
<tr>
<td></td>
<td>Macaroni penguin, <em>Eudyptes chrysocome filchali</em></td>
<td>Adult</td>
<td>New</td>
<td>2007</td>
<td>12</td>
<td>2.48 ± 0.35 (1.82–2.92)</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>Old</td>
<td>2006</td>
<td>12</td>
<td>2.09 ± 0.31 (1.65–2.51)</td>
<td>-19.6 ± 0.2d</td>
<td>9.1 ± 0.5c</td>
</tr>
<tr>
<td></td>
<td>Chick</td>
<td>New</td>
<td>2007</td>
<td>12</td>
<td>0.43 ± 0.10 (0.31–0.64)</td>
<td>-19.9 ± 0.2d</td>
<td>7.7 ± 0.6d</td>
</tr>
<tr>
<td>Gentoo penguin, <em>Pygoscelis papua</em></td>
<td>Adult</td>
<td>New</td>
<td>2007</td>
<td>11*</td>
<td>5.90 ± 1.91 (3.27–8.16)</td>
<td>3.1</td>
<td>-19.1 ± 0.2c</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>Old</td>
<td>2006</td>
<td>12</td>
<td>5.23 ± 1.12 (3.39–7.40)</td>
<td>-19.7 ± 0.6d</td>
<td>9.7 ± 0.7c</td>
</tr>
<tr>
<td></td>
<td>Chick</td>
<td>New</td>
<td>2007</td>
<td>12</td>
<td>1.85 ± 0.46 (1.29–2.88)</td>
<td>-17.9 ± 0.2D</td>
<td>11.3 ± 0.4C</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>New</td>
<td>2007</td>
<td>12</td>
<td>1.82 ± 0.30 (1.42–3.34)</td>
<td>5.4</td>
<td>-17.4 ± 0.2e</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>Old</td>
<td>2006</td>
<td>12</td>
<td>2.10 ± 0.36 (1.56–2.86)</td>
<td>-17.4 ± 0.2e</td>
<td>9.6 ± 0.4c</td>
</tr>
<tr>
<td></td>
<td>Chick</td>
<td>New</td>
<td>2007</td>
<td>15</td>
<td>0.34 ± 0.07 (0.25–0.46)</td>
<td>-17.4 ± 0.2e</td>
<td>9.6 ± 0.4c</td>
</tr>
</tbody>
</table>

* One outlier individual excluded for Hg: 16.85 \( \mu g \cdot g^{-1} \cdot dw \).

* Groups with the same letter are not statistically different (Tukey’s HSD, \( p < 0.05 \)). Upper and lower-case letters are for adults and chicks, respectively.

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**Fig. 1.** Boxplots of feather Hg concentrations in adults of six penguin species from the French Austral Territories in 2007. All the six species are offshore pelagic foragers; the inshore feeder gentoo penguin is not included in the figure (Table 1).
Table 3
AICc model ranking of latitudinal and historical feather Hg concentrations in penguin species from the French Austral Territories. Models are GLM with a gamma distribution and an inverse link function. Abbreviations: AICc, Akaike’s Information Criteria adjusted for small sample sizes values; wi, AICc weights.

<table>
<thead>
<tr>
<th>Models</th>
<th>k</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>wi</th>
<th>Exp. var.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Latitudinal variation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adults, GLM, Gamma distribution, Inverse Link Function, N = 86</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species + δ13C</td>
<td>8</td>
<td>108</td>
<td>0</td>
<td>0.66</td>
<td>0.89</td>
</tr>
<tr>
<td>Species + δ13C + δ15N</td>
<td>9</td>
<td>110</td>
<td>1.35</td>
<td>0.34</td>
<td>0.89</td>
</tr>
<tr>
<td>Species + δ13C + species:δ15N</td>
<td>14</td>
<td>119</td>
<td>10.3</td>
<td>0</td>
<td>0.90</td>
</tr>
<tr>
<td>Species</td>
<td>7</td>
<td>127</td>
<td>18.7</td>
<td>0</td>
<td>0.86</td>
</tr>
<tr>
<td>Species + δ13C</td>
<td>8</td>
<td>129</td>
<td>20.7</td>
<td>0</td>
<td>0.86</td>
</tr>
<tr>
<td>Null</td>
<td>1</td>
<td>288</td>
<td>181</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chicks, GLM, Gamma distribution, Inverse Link Function, N = 83</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species + δ13C + species:δ15C</td>
<td>14</td>
<td>107</td>
<td>0</td>
<td>0.90</td>
<td>0.95</td>
</tr>
<tr>
<td>Species + δ13C</td>
<td>8</td>
<td>-101</td>
<td>5.65</td>
<td>0.05</td>
<td>0.94</td>
</tr>
<tr>
<td>Species + δ15N</td>
<td>8</td>
<td>-99</td>
<td>7.65</td>
<td>0.02</td>
<td>0.94</td>
</tr>
<tr>
<td>Species + δ13C + δ15N</td>
<td>9</td>
<td>-99</td>
<td>8.17</td>
<td>0.02</td>
<td>0.94</td>
</tr>
<tr>
<td>Species</td>
<td>7</td>
<td>-98</td>
<td>9.22</td>
<td>0.01</td>
<td>0.94</td>
</tr>
<tr>
<td>Null</td>
<td>1</td>
<td>126</td>
<td>232</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Historical variation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adults, GLM, Gamma distribution, Inverse Link Function, N = 124</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>δ13C + δ15N + epoch + species</td>
<td>14</td>
<td>201</td>
<td>0</td>
<td>1</td>
<td>0.63</td>
</tr>
<tr>
<td>δ13C + δ15N + epoch + δ13C:epoch + species:epoch</td>
<td>5</td>
<td>279</td>
<td>78</td>
<td>0</td>
<td>0.63</td>
</tr>
<tr>
<td>δ13C + δ15N + epoch + δ13C:epoch + δ15N:epoch</td>
<td>6</td>
<td>281</td>
<td>80</td>
<td>0</td>
<td>0.63</td>
</tr>
<tr>
<td>δ13C + δ15N + epoch + species + δ13C:epoch + δ15N:epoch</td>
<td>9</td>
<td>284</td>
<td>83</td>
<td>0</td>
<td>0.64</td>
</tr>
<tr>
<td>δ13C + δ15N + epoch + species + δ13C:epoch + δ15N:epoch</td>
<td>10</td>
<td>287</td>
<td>86</td>
<td>0</td>
<td>0.64</td>
</tr>
<tr>
<td>Null</td>
<td>1</td>
<td>397</td>
<td>196</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*a* Only the five best ranked and the null models are presented.

*b* Number of parameters.

*c* Explained variation calculated from deviance and adjusted depending on k and N.

3.2. Temporal variation in adult feather Hg concentrations and influence of species and feeding habits

3.2.1. Inter-annual variation
Adult feather Hg concentrations were similar in 2006 and 2007 in king and gentoo penguins (pairwise Wilcoxon comparisons, V = 50 and 16, p = 0.424 and 0.148, respectively). On the other hand, feather Hg concentrations were higher in 2007 than in 2006 in Adélie, macaroni and southern rockhopper penguins (V = 2, 3 and 10, p = 0.006, 0.002 and 0.045, respectively), while they were lower in 2007 than 2006 in northern rockhopper penguins (V = 67, p = 0.027). Feather Hg concentrations were highly and positively correlated between the 2 years at the individual level (pooled data from six species; Pearson correlation, r = 0.93, p < 0.0001, n = 69, Fig. 2).

3.2.2. Historical variation
Hg and stable isotope values were measured in a total of 62 museum specimens of five penguin species dating back to the 50s–70s, and compared to contemporary 2007 values (see Table S1 in the Supplementary Information, SI). Feather Hg concentrations did not show a consistent temporal trend among species. In Antarctica, Adélie penguins had lower feather Hg concentrations in 2007 than in the 50s (Wilcoxon comparisons, W = 50, p = 0.001, Fig. 3), while emperor penguins had similar concentrations (W = 97, p = 0.888, Fig. 3). Conversely, at Crozet Islands, macaroni and gentoo penguins had higher feather Hg concentrations in 2007 than in the 70s (W = 36 and 10, p = 0.002 and p < 0.001, respectively, Fig. 4), whereas king penguins had unchanged burdens (W = 65, p = 0.157, Fig. 4). However, δ13C and δ15N values did not show a consistent temporal trend either (see Table S1 in the SI). Notably, δ15N values were significantly lower in 2007 than in the historical samples in emperor and macaroni penguins (Welsh two sample t test, t = 2.3 and 2.4, p = 0.034 and 0.033, respectively, Figs. 3 and 4).

Multifactorial analyses indicated that the species, epoch and the δ13C and δ15N signatures all were significant in explaining variation in feather Hg concentrations, with the maximal model having the best fit to the data (Table 3).
4. Discussion

The present study documents new information on Hg bio-accumulation in penguins from different latitudes of the Southern Ocean, including the first data on feather Hg concentrations of northern rockhopper penguins. Importantly, and with the only exception of king penguins (Scheiﬂer et al., 2005), this is the first report of historical feather Hg concentrations in seabirds from the southern Indian Ocean, revealing contrasting temporal trends depending on species.

4.1. Comparison to other penguin populations throughout the Southern Hemisphere

As useful bioindicators of Hg contamination in their food webs, penguins are the target of a growing number of studies investigating their pattern of contamination all around the Southern Hemisphere (e.g., Carravieri et al., 2013; Brasso et al., 2015; Pedro et al., 2015). Feather Hg concentrations were previously investigated in king penguins at the Crozet Islands (Scheiﬂer et al., 2005), revealing comparable results to the present study. Hg concentrations and inter-speciﬁc differences of the Crozet penguin community were remarkably similar to those of Kerguelen Islands (southern Indian Ocean), where the same four species breed (Carravieri et al., 2013; Bievin et al., 2013). Overall, feather Hg concentrations of TAAF penguins were comparable to previous studies from the Atlantic and Paciﬁc sectors of the Southern Ocean (e.g., Brasso et al., 2015), with Eudyptes and Pygoscelis penguins having usually lower feather Hg burdens than Aptenodytes penguins, likely because they feed at lower trophic levels (Becker et al., 2002; Carravieri et al., 2013; see below). King and macaroni penguins had similar or slightly lower feather Hg concentrations to those of South Georgia (southern Atlantic Ocean) populations (Table S2 in the SI), and both emperor and Adélie penguins at Terre...
Adélie had similar or slightly higher feather Hg concentrations than those reported at other Antarctic sites (Bargagli et al., 1998; Table S2 in the SI). A remarkable exception to this general pattern is that of the single inshore forager, the gentoo penguin (genus *Pygoscelis*), which, at Crozet Islands, had the highest mean feather Hg concentrations ever reported in penguins. This high Hg burden is similar to that of the Cape Estacade subpopulation at the Kerguelen Islands (Carravieri et al., 2013; Table S2 in the SI), therefore indicating the high quantity of Hg contained in the prey of gentoo penguins at both subantarctic sites, i.e. a mixture of benthic and demersal fish and of pelagic crustaceans from coastal environments (Ridoux, 1994; Lescroël et al., 2004; Cherel and Hobson, 2007, Table 1). By contrast, gentoo penguin feather Hg concentrations were lower at other subantarctic and Antarctic sites (Table S2 in the SI), where the species relies more largely on pelagic low-trophic level prey (e.g., Antarctic krill *Euphausia superba*, Brasso et al., 2014, Pedro et al., 2015) with lower Hg concentrations compared to benthic fish (e.g., Goutte et al., 2015). The high variability in feather Hg concentrations of gentoo penguins is thus likely linked to the variability of feeding habits of the species both at the individual and population levels (Lescroël et al., 2004; Miller et al., 2009; Carravieri et al., 2013).

### 4.2. Influence of age class, species and feeding habits on feather Hg burdens

Feather Hg burdens were lower in chicks than in adults in all the seven species studied, as previously shown in other penguins (Bargagli et al., 1998; Carravieri et al., 2013) and seabirds (e.g., Carravieri et al., 2014a; Catry et al., 2008; Bond and Diamond, 2009). Lower feather Hg concentrations in chicks have been attributed to their short period of exposure (the chick-rearing period) when compared to adults (inter-moult period, ~ one year) (Burger and Gochfeld, 2004). Hence, the duration of the chick-rearing period influences the adult to chick ratio in Hg burdens (Furness et al., 1990), i.e., species having long chick-rearing periods have low ratios, as shown in king penguins (Carravieri et al., 2013; this study, Table 2). Nevertheless, variations of feather Hg levels in adults and chicks are also driven by age class differences in diet (Carravieri et al., 2014a), since in some species adults feed their chicks with different prey than those they consume themselves, both during the breeding and non-breeding periods (e.g., Wilson et al., 2004; Cherel, 2008).

For both adults and chicks, the species was the main factor explaining variation in feather Hg levels (Table 3). The species indeed integrates physiological and ecological traits that may drive variation in Hg exposure and bioaccumulation (e.g., Anderson et al., 2009). However, the main factors explaining inter-species differences in Hg burdens are feeding habits, rather than physiological or taxonomic differences (Stewart et al., 1999; Anderson et al., 2009; Carravieri et al., 2014a). Given Hg biomagnification in the marine environment (Chen et al., 2008; Mason et al., 2012), high trophic level prey, such as fish and cephalopods, show higher Hg concentrations than planktonic organisms (Bustamante et al., 2006;
Kojadinovic et al., 2006; Stewart et al., 1997), resulting in higher Hg transfer to their consumers. As expected, species feeding mainly on fish had higher feather Hg concentrations than those relying mainly on crustaceans, both at the Antarctic and subantarctic sites, and among both adults and chicks (Fig 1 and Tables 1 and 2).

Namely, the Antarctic emperor penguins that feed extensively on pelagic fish had higher feather Hg concentrations than the sympatric Adélie penguins that rely mainly on crustaceans. Similarly, subantarctic king penguins that prey almost exclusively on myctophid fish had higher Hg burdens than the sympatric southern rockhopper penguins that rely on pelagic crustaceans.

Hence, the trophic position (inferred from δ13N values) had a significant effect in explaining both geographical and temporal variation in feather Hg concentrations of adults (Table 2). The δ13C value, on the other hand, had strong statistical power in explaining variation in Hg burdens in chicks. This indicates that penguin chicks were exposed to different Hg quantities depending on the habitats used by their parents to capture prey. The weak effect of feeding habitat on adult feather Hg concentrations is likely linked to the slight temporal uncoupling of stable isotopes and Hg integration in adult feathers (e.g., Thompson et al., 1998), with Hg concentrations reflecting accumulated burdens over the breeding, pre-moulting and wintering periods. While the habitat inferred from δ13C signatures indicate the pre-moulting feeding habitat only (e.g., Cherel et al., 2005). This result also highlights that chicks may be more suitable as local biomonitor of Hg accumulation than adults, because their exposure can be better ascertained both spatially and temporally (Becker et al., 1993; Burger and Goichfeld, 2004).

4.3. Latitudinal variation within the southern Indian Ocean

The present study shows that Antarctic species had lower feather Hg concentrations than subantarctic and subtropical species (Fig 1), irrespective of age class and dietary group. Namely, among mainly crustacean-eating species, Adélie penguins that feed in pelagic Antarctic waters year-round (Table 1), had less than half the feather Hg concentrations of the subantarctic southern rockhopper penguins. Similarly, among mainly fish-eaters, emperor penguins, which rely on Antarctic waters, had lower Hg concentrations than king penguins that forage almost exclusively in oceanic waters close to the Polar Front. Geographical variations in penguin Hg burdens seem thus to derive from differential Hg transfer to food webs depending on the water mass, rather than from dietary differences. Such a geographical variation has not been shown in other penguin populations from around the Southern Hemisphere (Table S2 in the SI). Nevertheless, a similar trend was shown in other penguin populations from around the Southern Hemisphere (Table S2 in the SI), suggesting contemporaneous groups; and 2) while Adélie and macaroni penguins showed a temporal increase in new feathers (2007) (Fig. 2), especially in species with high intra-specific variation in Hg concentrations (gentoo penguins). This corroborates the results from previous studies (Schei er et al., 2005; Carravieri et al., 2013; Brasso et al., 2014). In the present study only two (king and gentoo) out of six penguin species showed no inter-annual variation in feather Hg concentrations, while the other species showed a temporal increase (Adélie, macaroni and southern rockhopper) or decrease (northern rockhopper penguins) between 2006 and 2007. Since no inter-annual variation in feather stable isotopes values was reported in these individuals (Jaeger and Cherel, 2011), the temporal change in Hg exposure is likely due to environmental factors rather than to a dietary change. This is supported by the fact that at the individual level, Hg exposure was similar in both years, i.e., individuals with a high feather Hg concentration in old feathers (2006), had also high concentrations in new feathers (2007) (Fig. 2), especially in species with high intra-specific variation in Hg concentrations (gentoo penguins). This corroborates the results from previous studies (Schei er et al., 2005; Carravieri et al., 2013) suggesting that individuals may vary in their ability to excrete Hg in their feathers and/or that individuals have consistent feeding strategies (and thus Hg exposure) over the long-term, as previously shown in other seabirds (e.g., Ceia et al., 2012).

4.4.2. Historical variation

Since Hg is strongly bound to feather keratin, resisting vigorous chemical treatments (Appelquist et al., 1984), archives of feather samples from museum skins have served widely to investigate long-term temporal trends in seabird Hg exposure. Significant increases in feather Hg concentrations have been detected in seabirds from the Arctic (e.g., Muir et al., 1999; Dietz et al., 2009; Bond et al., 2015), North Atlantic (Thompson et al., 1992; Monteiro and Furness, 1997) and Pacific (Vo et al., 2011) Oceans during the last century. Here, contrary to our prediction, long-term temporal variation in feather Hg concentrations in Southern Ocean penguins did not indicate a consistent increase, but instead varied in different ways depending on species. Notably, in Antarctica, no change in penguin Hg bioaccumulation can be clearly ruled out, since 1) feather Hg concentrations in emperor penguins were similar in the 50s and contemporary groups; and 2) while Adélie penguins showed a significant decrease between the two time periods (~77%), the small sample size and the high between-individual variation of the historical samples call for caution in the interpretation (Fig. 3). Conversely, results in subantarctic species offer a different picture. Namely, macaroni and gentoo penguins had significantly higher
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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.02.010.

References


