



# Penguins as bioindicators of mercury contamination in the southern Indian Ocean: geographical and temporal trends<sup>☆</sup>



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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 ( $\delta^{13}\text{C}$ , habitat;  $\delta^{15}\text{N}$ , diet/trophic level). Adult feather Hg concentrations in contemporary individuals ranged from  $0.7 \pm 0.2$  to  $5.9 \pm 1.9 \mu\text{g g}^{-1}$  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, Me-Hg), Hg bioaccumulates in the tissues of marine organisms and biomagnifies 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 bioaccumulation in marine biota, such as marine mammals and

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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 (Thompson et al., 1998), and are commonly used for seabird Hg biomonitoring over the short- and long-term (Burger and Gochfeld, 2004). 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 subantarctic, to subtropical waters (Blévin et al., 2013; Carravieri et al., 2014b; Goutte et al., 2014). This trend should however be confirmed by multi-sites and multi-species studies, and discussed in the light of the pioneer data on Me–Hg distribution in Southern Ocean 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 ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ), 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 (Blévin 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 *sensu 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 *Aptenodytes forsteri* and Adélie *Pygoscelis adeliae* penguins at Adélie land; king *Aptenodytes patagonicus*, macaroni *Eudyptes chrysolophus*, southern rockhopper *Eudyptes chrysolophus filholi* and gentoo penguins *Pygoscelis papua* at Crozet Islands; and northern rockhopper penguins *Eudyptes 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 moults 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).

**Table 1**  
Foraging ecology of penguins during the breeding and non-breeding periods at three sites of the French Antarctic Territories (TAAF).

Species	Foraging habitat		Chick diet (Main prey group)	References
	Breeding season (Horizontal; vertical)	Non-breeding season (Horizontal; vertical)		
<b>Adélie land (Antarctica)</b>				
Emperor penguin	Antarctic waters (offshore; pelagic)	Antarctic waters (offshore; pelagic)	Pelagic fish	Offredo and Ridoux (1986); Cherel (2008); Zimmer et al. (2008)
Adélie penguin	Antarctic waters (offshore; pelagic)	Antarctic waters (offshore; pelagic)	Pelagic crustaceans (fish)	Ridoux and Offredo (1989); Wienecke et al. (2000); Cherel (2008)
<b>Crozet Islands (Subantarctic Zone)</b>				
King penguin	Polar Frontal Zone (offshore; pelagic)	Southern Ocean (offshore; pelagic)	Mesopelagic fish	Ridoux (1994); Cherel et al. (2007); Bost et al. (2009)
Macaroni penguin	Crozet waters (offshore; pelagic)	Subantarctic waters and Polar Front (offshore; pelagic)	Pelagic crustaceans (fish)	Ridoux (1994); Thiébot et al. (2011a,b)
Southern rockhopper penguin	Crozet waters (offshore; pelagic)	Subantarctic waters and Polar Front (offshore; pelagic)	Pelagic crustaceans	Ridoux (1994); Tremblay and Cherel (2003); Cherel et al. (2007); Thiébot et al. (2012)
Gentoo penguin	Crozet waters (inshore; benthic and pelagic)	Crozet waters (inshore; benthic and pelagic)	Crustaceans and fish	Ridoux (1994)
<b>Amsterdam Island (Subtropical Zone)</b>				
Northern rockhopper penguin	Amsterdam waters (offshore; pelagic)	Subtropical waters (offshore; pelagic)	Pelagic crustaceans (squid)	Tremblay and Cherel (2003); Thiébot et al. (2012)

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:  $0.27 \pm 0.06 \mu\text{g g}^{-1}$  dry mass) was used to check accuracy. Our measured values were  $0.23 \pm 0.003 \mu\text{g g}^{-1}$  dry mass,  $n = 7$ . Blanks were analysed before each set of samples. The detection limit of the method was  $0.005 \mu\text{g g}^{-1}$  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 ( $\sim 0.3$  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  $\delta$  notation relative to Vienna Pee Dee Belemnite and atmospheric  $\text{N}_2$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively. Replicate measurements of internal laboratory standards (acetanilide) indicated measurement errors < 0.15‰ for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. Results of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  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  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  than background carbon, Keeling, 1979) and second, the effect of the increase in atmospheric  $\text{CO}_2$  and thus aqueous  $\text{CO}_2$ , which increases phytoplankton fractionation, reducing its  $\delta^{13}\text{C}$  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,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  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 parameters in describing geographical and temporal variation in feather Hg concentrations. The models were restricted to primary effects (explanatory variables: species,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and/or epoch) and two-way interactions. Feather  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  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  $\alpha < 0.05$  was used for all tests, both in unifactorial and multifactorial analysis. Values are means  $\pm$  SD.

## 3. Results

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

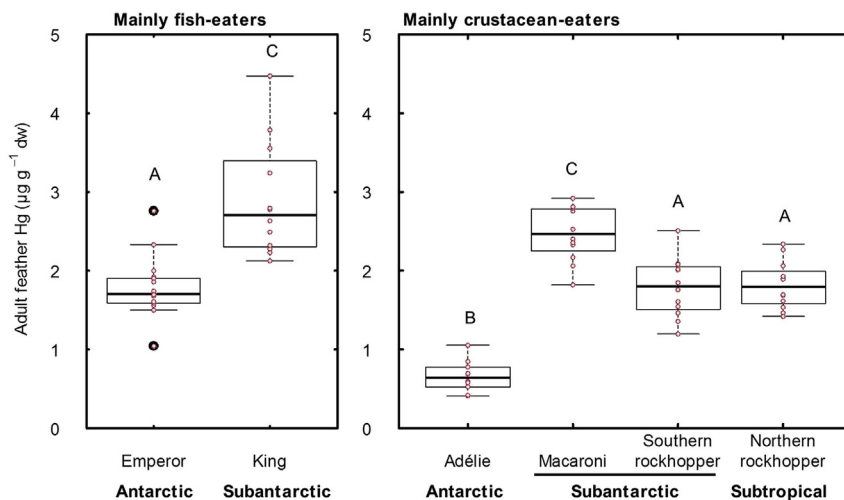
Feather Hg and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were measured in 170 individual penguins, including adults and chicks, during the 2006–2007 austral summer season. Among adult penguins, there was a 9-fold difference in Hg concentrations between the least and the most contaminated species, namely the Adélie and gentoo penguins, respectively (Table 2). Based on 2007 feathers, interspecific 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 subtropical species, irrespective of the dietary group (mainly fish- or mainly crustacean-feeders, Fig. 1). Feather  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values differed widely among adult penguins (ANOVA,  $F_{6,79} = 399$  and 68,

**Table 2**  
Feather Hg concentrations ( $\mu\text{g g}^{-1}$  dw) and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (‰) of seven penguin species in 2006 and 2007. Values are means  $\pm$  SD with ranges in parentheses for Hg.

Species age class	Feather	Year	n	Hg <sup>a</sup>	Adult:Chick Hg ratio	$\delta^{13}\text{C}^a$	$\delta^{15}\text{N}^a$
<b>Adélie land (Antarctica)</b>							
Emperor penguin, <i>Aptenodytes forsteri</i>							
Adult	Old	2007	17	1.77 $\pm$ 0.37 (1.05–2.76) <b>A</b>	2.9	–23.1 $\pm$ 0.3 <b>A</b>	12.1 $\pm$ 0.3 <b>A</b>
Chick	New	2007	12	0.61 $\pm$ 0.11 (0.44–0.86) <b>a</b>		–24.2 $\pm$ 0.3 <b>a</b>	12.4 $\pm$ 0.4 <b>a</b>
Adélie penguin, <i>Pygoscelis adeliae</i>							
Adult	New	2007	10	0.66 $\pm$ 0.20 (0.41–1.06) <b>B</b>	3.5	–23.4 $\pm$ 0.4 <b>A</b>	10.7 $\pm$ 0.6 <b>B</b>
Adult	Old	2006	12	0.43 $\pm$ 0.13 (0.24–0.72)			
Chick	New	2007	10	0.19 $\pm$ 0.06 (0.07–0.27) <b>b</b>		–23.5 $\pm$ 0.2 <b>b</b>	10.7 $\pm$ 0.4 <b>b</b>
<b>Crozet Islands (SubantarcticZone)</b>							
King penguin, <i>Aptenodytes patagonicus</i>							
Adult	New	2007	12	2.89 $\pm$ 0.73 (2.13–4.47) <b>C</b>	1.6	–20.8 $\pm$ 0.6 <b>B</b>	11.3 $\pm$ 0.5 <b>B,C</b>
Adult	Old	2006	12	2.94 $\pm$ 0.47 (2.15–3.63)			
Chick	New	2007	12	1.80 $\pm$ 0.24 (1.33–2.11) <b>c</b>		–21.6 $\pm$ 0.3 <b>c</b>	10.5 $\pm$ 0.4 <b>b</b>
Macaroni penguin, <i>Eudyptes chrysolophus</i>							
Adult	New	2007	12	2.48 $\pm$ 0.35 (1.82–2.92) <b>C</b>	5.8	–21.1 $\pm$ 0.3 <b>B</b>	9.7 $\pm$ 0.3 <b>D</b>
Adult	Old	2006	12	2.09 $\pm$ 0.31 (1.65–2.51)			
Chick	New	2007	12	0.43 $\pm$ 0.10 (0.31–0.64) <b>d</b>		–19.6 $\pm$ 0.2 <b>d</b>	9.1 $\pm$ 0.5 <b>c</b>
Southern rockhopper penguin, <i>Eudyptes chrysocome filholi</i>							
Adult	New	2007	12	1.79 $\pm$ 0.37 (1.20–2.51) <b>A</b>	5.3	–21.1 $\pm$ 0.2 <b>B</b>	8.9 $\pm$ 0.4 <b>E</b>
Adult	Old	2006	12	1.62 $\pm$ 0.35 (1.26–2.51)			
Chick	New	2007	12	0.34 $\pm$ 0.05 (0.25–0.43) <b>d</b>		–19.9 $\pm$ 0.2 <b>d</b>	7.7 $\pm$ 0.6 <b>d</b>
Gentoo penguin, <i>Pygoscelis papua</i>							
Adult	New	2007	11*	5.90 $\pm$ 1.91 (3.27–8.16) <b>D</b>	3.1	–19.1 $\pm$ 0.2 <b>C</b>	11.1 $\pm$ 0.8 <b>B,C</b>
Adult	Old	2006	12	5.23 $\pm$ 1.12 (3.39–7.40)			
Chick	New	2007	12	1.88 $\pm$ 0.46 (1.29–2.88) <b>c</b>		–19.7 $\pm$ 0.6 <b>d</b>	9.7 $\pm$ 0.7 <b>c</b>
<b>Amsterdam Island (Subtropical Zone)</b>							
Northern rockhopper penguin, <i>Eudyptes moseleyi</i>							
Adult	New	2007	12	1.82 $\pm$ 0.30 (1.42–3.34) <b>A</b>	5.4	–17.9 $\pm$ 0.2 <b>D</b>	11.3 $\pm$ 0.4 <b>C</b>
Adult	Old	2006	12	2.10 $\pm$ 0.36 (1.56–2.86)			
Chick	New	2007	15	0.34 $\pm$ 0.07 (0.25–0.46) <b>d</b>		–17.4 $\pm$ 0.2 <b>e</b>	9.6 $\pm$ 0.4 <b>c</b>

\* One outlier individual excluded for Hg: 16.85  $\mu\text{g g}^{-1}$  dw.

<sup>a</sup> 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.



**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).

respectively, both  $p < 0.0001$ ), with each species having a unique isotopic niche (Table 2). Multifactorial analyses showed that the species and the  $\delta^{15}\text{N}$  signature had the greatest explanatory power of adult feather Hg concentrations at the different sites, while  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, respectively, both  $p < 0.0001$ ), with the exception of macaroni and gentoo penguin chicks at Crozet Islands, which had similar feather  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (Table 2). Multifactorial analyses revealed that, in contrast to adults, the  $\delta^{13}\text{C}$  signature was as important as the species in explaining variation of chick feather Hg concentrations (Table 3).



**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.

Models <sup>a</sup>	$k^b$	AIC <sub>c</sub>	$\Delta\text{AIC}_c^c$	$w_i^d$	Exp. var. <sup>e</sup>
<b>Latitudinal variation</b>					
Adults, GLM, Gamma distribution, Inverse Link Function, $N = 86$					
Species + $\delta^{15}\text{N}$	8	108	0	0.66	0.89
Species + $\delta^{15}\text{N}$ + $\delta^{13}\text{C}$	9	110	1.35	0.34	0.89
Species + $\delta^{15}\text{N}$ + species: $\delta^{15}\text{N}$	14	119	10.3	0	0.90
Species	7	127	18.7	0	0.86
Species + $\delta^{13}\text{C}$	8	129	20.7	0	0.86
Null	1	288	181	0	0
Chicks, GLM, Gamma distribution, Inverse Link Function, $N = 83$					
Species + $\delta^{13}\text{C}$ + species: $\delta^{13}\text{C}$	14	-107	0	0.90	0.95
Species + $\delta^{13}\text{C}$	8	-101	5.65	0.05	0.94
Species + $\delta^{15}\text{N}$	8	-99	7.65	0.02	0.94
Species + $\delta^{13}\text{C}$ + $\delta^{15}\text{N}$	9	-99	8.17	0.02	0.94
Species	7	-98	9.22	0.01	0.94
Null	1	126	232	0	0
<b>Historical variation</b>					
Adults, GLM, Gamma distribution, Inverse Link Function, $N = 124$					
$\delta^{13}\text{C}$ + $\delta^{15}\text{N}$ + epoch + species + $\delta^{13}\text{C}$ :epoch + $\delta^{15}\text{N}$ :epoch + species:epoch	14	201	0	1	0.83
$\delta^{13}\text{C}$ + $\delta^{15}\text{N}$ + epoch + $\delta^{13}\text{C}$ :epoch	5	279	78	0	0.63
$\delta^{13}\text{C}$ + $\delta^{15}\text{N}$ + epoch + $\delta^{13}\text{C}$ :epoch + $\delta^{15}\text{N}$ :epoch	6	281	80	0	0.63
$\delta^{13}\text{C}$ + $\delta^{15}\text{N}$ + epoch + species + $\delta^{13}\text{C}$ :epoch	9	284	83	0	0.64
$\delta^{13}\text{C}$ + $\delta^{15}\text{N}$ + epoch + species + $\delta^{13}\text{C}$ :epoch + $\delta^{15}\text{N}$ :epoch	10	287	86	0	0.64
Null	1	397	196	0	0

<sup>a</sup> Only the five best ranked and the null models are presented.

<sup>b</sup> Number of parameters.

<sup>c</sup> Scaled  $\Delta\text{AIC}_c$ ;  $\Delta\text{AIC}_c = 0.00$  is interpreted as the best fit to the data among the models.

<sup>d</sup> Weight of evidence interpreted as a proportion. Weights across all models sum to 1.00.

<sup>e</sup> Explained variation calculated from deviance and adjusted depending on  $k$  and  $N$ .

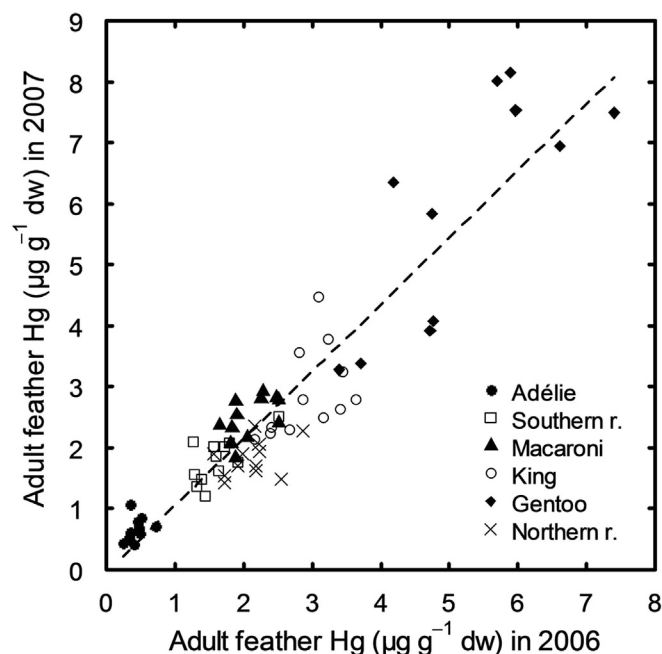
### 3.2. Temporal variation in adult feather Hg concentrations and influence of species and feeding habits

#### 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). Feather  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values did not show a consistent temporal trend either (see Table S1 in the SI). Notably,  $\delta^{15}\text{N}$  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).



**Fig. 2.** Positive correlation between feather Hg concentrations ( $\mu\text{g g}^{-1} \text{dw}$ ) of individual adult penguins during two consecutive years (2006 and 2007) from different sites of the French Austral Territories.

Multifactorial analyses indicated that the species, epoch and the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures all were significant in explaining variation in feather Hg concentrations, with the maximal model having the best fit to the data (Table 3).

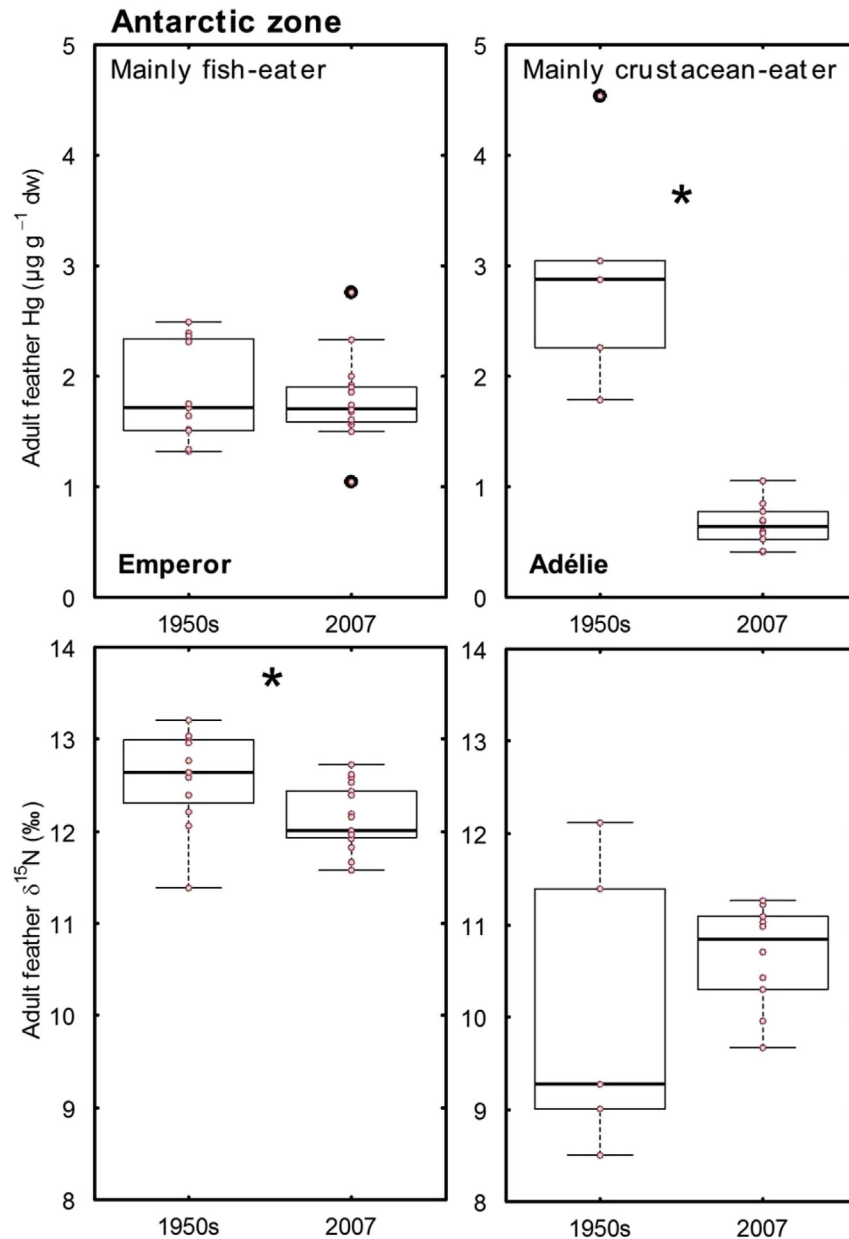


Fig. 3. Boxplots of feather Hg concentrations ( $\mu\text{g g}^{-1} \text{dw}$ ) and  $\delta^{15}\text{N}$  values (‰) in Antarctic penguin species from the French Austral Territories in the 1950s and in 2007.

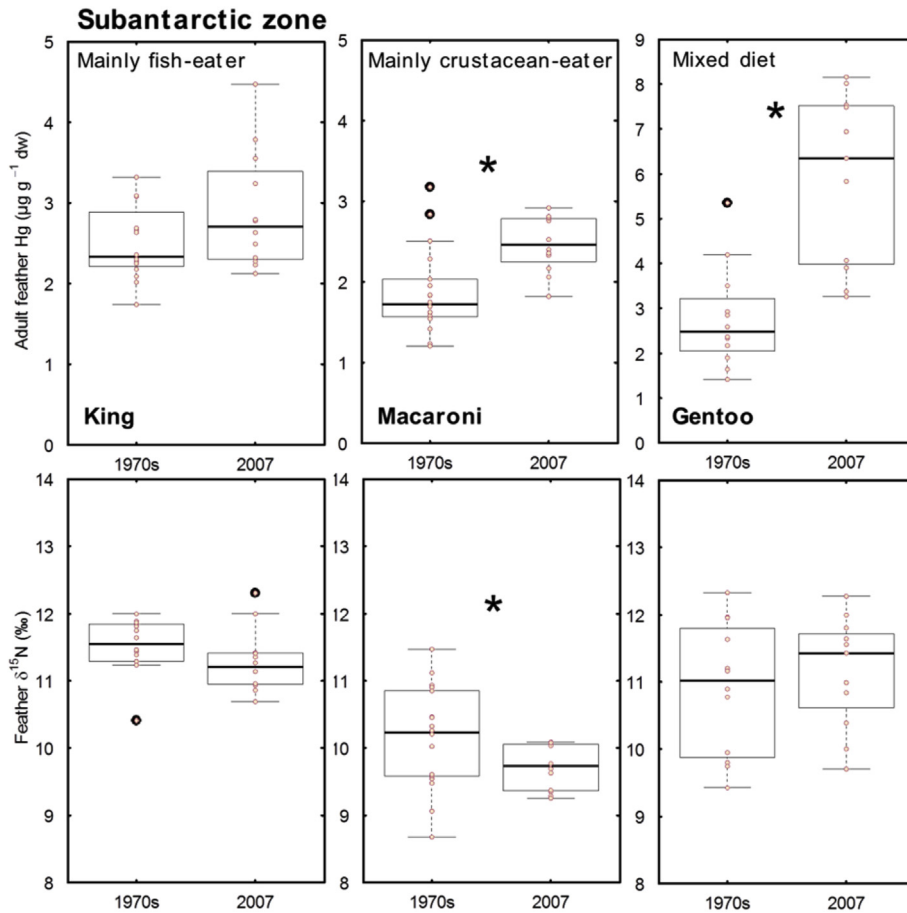
#### 4. Discussion

The present study documents new information on Hg bioaccumulation 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; Blévin 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



**Fig. 4.** Boxplots of feather Hg concentrations ( $\mu\text{g g}^{-1}$  dw) and  $\delta^{15}\text{N}$  values (‰) in subantarctic penguin species from the French Austral Territories in the 1970s and in 2007. Note the different axis range for feather Hg concentrations in gentoo penguins.

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  $\delta^{15}\text{N}$  values) had a significant effect in explaining both geographical and temporal variation in feather Hg concentrations of adults (Table 2). The  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  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 biomonitors of Hg accumulation than adults, because their exposure can be better ascertained both spatially and temporally (Becker et al., 1993; Burger and Gochfeld, 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 previously reported at the Kerguelen Islands, where oceanic seabird species feeding in cold Antarctic waters had lower feather Hg concentrations than those relying on warmer northern waters (Blévin et al., 2013; Carravieri et al., 2014a). Surprisingly, the only biogeochemical survey on Hg speciation and distribution in the Southern Ocean has shown that Antarctic waters are enriched with Me-Hg, the bioavailable form that is readily transferred to food webs, when compared to subantarctic waters (Cossa et al., 2011). Lower Hg burdens in Antarctic than subantarctic predators are therefore contradictory. Yet, several biotic and abiotic factors influence Hg bioavailability and food web transfer, including atmospheric Hg deposition rate, oxygen and organic matter concentrations, microbial activity, temperature (e.g., Fitzgerald et al., 2007) and, importantly, food web structure (Cabana and Rasmussen, 1994; Point et al., 2011; Braune et al., 2014). An increasing number of trophic levels between predators and their prey thus results in higher Hg concentrations in the predators' tissues (Cabana et al., 1994). Antarctic food webs are rather simple,

with upper predators relying directly or indirectly on a few key species, such as the Antarctic krill and Antarctic silverfish (e.g., Corsolini, 2009). Conversely, predators in subantarctic food webs feed on a large variety of prey species (e.g., Cherel et al., 2007). A weaker biomagnification of Hg due to a lower number of trophic levels, despite relatively high Me-Hg concentrations in the water (Cossa et al., 2011), could explain the low Hg burden in Antarctic seabirds.

#### 4.4. Temporal variation within the southern Indian Ocean

##### 4.4.1. Inter-annual variation

In the marine environment, the quantity of Hg available to predators may change annually as a consequence of differences in Hg deposition and *in situ* methylation rates, in phytoplankton intake and biomass, among other factors (Fitzgerald et al., 2007; Driscoll et al., 2013). Changes in feeding strategies or in the way Hg is transferred within food webs can also lead to annual differences in Hg concentrations in predators' tissues (Braune et al., 2014; Bond et al., 2015). Nevertheless, in remote marine regions that are subject to global rather than point sources of Hg, inter-annual differences in feather Hg contamination of seabirds have been found to be negligible (Scheifler 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 (Scheifler 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



feather Hg concentrations in present-day than in the 70s (+32% and +53%, respectively, Fig. 4). The same trend was true in king penguins, although not significantly so (+14%, Fig. 4, but see Scheiffler et al., 2005). While gentoo and king penguins had similar  $\delta^{15}\text{N}$  values at both time periods, macaroni penguins occupied a lower trophic position in 2007. Taking into account Hg bio-magnification, the detected increase in feather Hg concentrations of macaroni penguins is contradictory, and thus strongly suggests that it was linked to increasing Hg concentrations in the food web rather than to a dietary shift (Bond et al., 2015). Other subantarctic seabirds have been reported to have increasing feather Hg concentrations (i.e., black-browed *Thalassarche melanophrys* and grey-headed *T. chrysostoma* albatrosses, South Georgia, Becker et al., 2002), although potential temporal changes in feeding habits were not simultaneously assessed. In contrast to results from Northern Hemisphere biota, there is therefore a lack of a significant, consistent increase in Hg burdens in seabird species from the Southern Ocean in the past century (Thompson et al., 1993; Becker et al., 2002; Scheiffler et al., 2005; present work). This may indicate that Hg transfer to Southern Ocean predators has remained relatively constant in this time period, in accordance with the lower anthropogenic emissions and deposition rates that have characterised the Southern Hemisphere when compared to the Northern one (e.g., UNEP, 2013). Nevertheless, the increase detected here in subantarctic species may be indicative of recent changes of Hg emission and/or deposition patterns, linked to the development of Asian and Southern Hemisphere countries, as shown by increased Hg deposition in peat cores and lake sediments in the Southern Hemisphere (e.g., Hermanns and Biester, 2013). Since there is an important temporal lag before that changes in Hg atmospheric patterns are mirrored in Hg deposition, circulation and food web transfer in the ocean (Driscoll et al., 2013; UNEP, 2013), future increasing trends in marine biota from the Southern Ocean could be expected. Therefore, continuous monitoring is highly needed to confirm or not these trends in penguins, by using larger sample sizes and a larger time-window, and by taking into account potential dietary preference change over time (Braune et al., 2014; Emslie et al., 2014; Bond et al., 2015).

### Conflicts of interest

The authors declare that there are no conflicts of interest.

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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>.

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