Penguins as bioindicators of mercury contamination in the Southern Ocean: Birds from the Kerguelen Islands as a case study

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Abstract: Seabirds have been used extensively as bioindicators of mercury (Hg) contamination in the marine environment, although information on flightless species like penguins remains limited. In order to assess the use of penguins as bioindicators of Hg contamination in subantarctic and Antarctic marine ecosystems, Hg concentrations were evaluated in the feathers of the four species that breed on the Kerguelen Islands in the southern Indian Ocean. Compared to other seabirds, adult Kerguelen penguins had low to moderate feather Hg concentrations, with an average ranging from 1.96 ± 0.41 µg·g⁻¹ dry weight in the southern rockhopper to 5.85 ± 3.00 µg·g⁻¹ dry weight in gentoo penguins. The species was a major determinant of Hg contamination, with feather Hg concentrations being lower in the oceanic species (king and crested penguins) than in the coastal ones (gentoo penguins). In all species however, feather Hg concentrations were higher in adults than in chicks, reflecting the different periods of Hg bioaccumulation in the internal tissues of the two age classes. The relationship between adult penguin trophic ecology and Hg burdens was investigated using stable isotopes. Feeding habits (reflected by δ¹⁵N values) had a greater effect on adult feather Hg concentrations when compared to foraging habitats (reflected by δ¹³C values), indicating Hg biomagnification in Kerguelen neritic and oceanic waters. Dietary preferences were crucial in explaining individual feather Hg concentrations, as highlighted by intra-specific variation in Hg levels of gentoo penguins sampled at two different breeding sites of the Archipelago. Penguins appear to reflect Hg bioavailability reliably in their foraging environment and could serve as efficient bioindicators of Hg contamination in the Southern Ocean on different spatial and temporal scales.

Key words: Antarctica; Indian Ocean; seabird; trace element; metal; stable isotopes.
1. Introduction

While occurring naturally, mercury (Hg) is a pervasive environmental contaminant that negatively impacts humans and wildlife (Bond and Diamond, 2009; Scheuhammer et al., 2007). Over centuries a vast range of human activities have increased emissions to the atmosphere, modifying the cycling of Hg on the world scale (Fitzgerald et al., 2007; Selin, 2009). Aquatic environments, including marine ecosystems, are major repositories of natural and anthropogenic Hg. Hence, Hg is widely distributed in the World Ocean as a consequence of both long-range atmospheric transport and deposition (Ebinghaus et al., 2002; Fitzgerald et al., 1998). Despite being free of industrial sources of contamination and scarcely affected by local anthropogenic pollution, the Southern Ocean presents some unique features in the distribution of the different Hg species (Cossa et al., 2011), including elevated levels of contamination in some biota, especially top predators (Anderson et al., 2009; Bargagli et al., 1998; Muirhead and Furness, 1988). Indeed, Hg is known to bioaccumulate in the tissues of living organisms and to biomagnify within food webs, leaving top predators at risk of high contamination levels through food intake (Furness and Camphuysen, 1997; Morel et al., 1998). Top consumers include seabirds that have been identified as effective Hg sentinels in the marine environment (e.g., Burger and Gochfeld, 2004; Furness, 1993). In this context, seabird feathers have proved to be a valuable tissue, as they represent the main route of Hg excretion in birds (e.g., Braune and Gaskin, 1987; Monteiro and Furness, 1995) and can be easily collected during nesting from both chicks and breeding adults.

Previous investigations on Hg contamination of seabirds from the Southern Ocean essentially focused on flying species, mainly of the order Procellariiformes (Anderson et al., 2009; Becker et al., 2002; Bocher et al., 2003). By contrast, very little is known
about the Hg exposure of diving seabirds like penguins (Bargagli et al., 1998; Becker et al., 2002; Scheifler et al., 2005). Penguins are less Hg contaminated than some other seabirds, but they present interesting ecological and practical advantages over flying species to investigate Hg contamination within Antarctic and subantarctic food webs. Firstly, unlike most albatrosses and petrels that disperse in northern waters during the nonbreeding period (BirdLife International, 2004), Antarctic and subantarctic penguins are restricted to the Southern Ocean all year long (Ballard et al., 2010; Thiebot et al., 2012, 2011a, 2011b). Penguins are thus truly representative of the level of contamination of Antarctic and subantarctic ecosystems. Secondly, depending on species, penguins forage at different depths of the water column, namely the epi-, meso-pelagic and benthic zones that are known to present heterogeneous Hg concentrations and Hg species distributions (Cossa et al., 2011; Fitzgerald et al., 2007; Thompson et al., 1998). Thirdly, penguins renew their whole plumage annually over a 2-4 wks period on land (Adams and Brown, 1990; Cherel et al., 1994), thus contrasting with most other birds that present a prolonged, sequential moult leading to higher Hg concentrations in the earlier than in the later growing feathers (Furness et al., 1986). Hence, penguins appear to be good models to evaluate Hg contamination in their foraging environment, but this has yet to be proved conclusively.

The main objective of the present study was to assess the use of penguins as bioindicators of Hg contamination in marine ecosystems of the Southern Ocean. The following predictions were tested on the penguin assemblage from the subantarctic Kerguelen Islands where four species live in sympatry.

1) As already depicted in other seabirds (Anderson et al., 2009; Becker et al., 2002; Blévin et al., 2013), the diet and foraging ecology of penguins should play an important role in explaining feather Hg levels, because ingestion of food is the main
route of Hg exposure in birds. As Kerguelen penguin species display contrasted feeding ecology (Table 1), feather Hg concentrations should show important inter-specific differences. The respective effects of habitats and diets were tested by using the isotopic niche as a proxy of the trophic niche of the species, with the ratios of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) reflecting their foraging habitats and trophic positions, respectively. The isotopic method was already validated in the area, with seabird $\delta^{13}C$ values indicating their latitudinal foraging grounds and depicting offshore versus inshore consumers and their $\delta^{15}N$ values increasing with trophic level (Cherel and Hobson, 2007; Cherel et al., 2010; Cherel et al., 2007; Jaeger et al., 2010). Taking into account the penguins’ foraging ecology (Table 1), we make the following predictions. Firstly, feeding habitat ($\delta^{13}C$) should shape seabird Hg contamination, because Hg is not homogeneously distributed in marine ecosystems (Cossa et al., 2011; Hammerschmidt and Bowman, 2012). For example, benthic foragers should have higher feather Hg concentrations than pelagic foragers in relation to the substantial production of Me-Hg in coastal marine sediments (Fitzgerald et al., 2007). Secondly, penguins with the highest trophic positions ($\delta^{15}N$) should show the highest feather Hg concentrations, because Hg biomagnifies within marine food webs (e.g., Selin, 2009).

2) Between-year variation of Hg levels is an issue rarely investigated in seabirds. Taking into account that (i) penguin feeding habits are comparable in the breeding and non-breeding seasons (Thiebot et al., 2012, 2011a, 2011b); (ii) penguin feather Hg temporal integration is constant (one year); and (iii) the Kerguelen Islands are removed from point anthropogenic Hg sources; no variation in feather Hg levels should be detected on a short temporal scale (two following years).

3) As already found in birds (Burger, 1993), feather Hg concentrations should be higher in adult penguins than in chicks at fledging, mainly because: (i) the time interval
of Hg accumulation is longer in adults than in chicks (~12 months of inter-moult period for adults and the 3-9 months of rearing period in chicks, depending on species) and (ii) Hg can bioaccumulate in internal tissues of long-lived animals over their whole life span.

The present article is the first exploratory step of a wider investigation on penguins as bioindicators of Hg contamination in the Southern Ocean. In the second step, we will focus on penguins breeding at different locations in order to highlight potential geographic variation of Hg levels over a large latitudinal gradient, from the subtropics to Antarctica. In the third and final step, we will assess historical trends of penguin Hg contamination by comparing actual feather concentrations with those of museum specimens dating as far back as the 1950s.

2. Materials and methods

2.1. Study site, species and field collections

Fieldwork was carried out during the 2006–2007 austral summer on the Kerguelen Islands (49°21’S, 70°18’E), which are located in the southern part of the Polar Frontal Zone, in the immediate vicinity of the Polar Front (Park and Gamberoni, 1997). The Kerguelen assemblage of penguins is composed of four species, namely the king Aptenodytes patagonicus (KP), macaroni Eudyptes chrysolophus (MP), southern rockhopper Eudyptes chrysocome filholi (SRP) and gentoo penguins Pygoscelis papua (GP) (Table 1). Sampling was conducted at different locations of the archipelago, depending on the species breeding sites. Since GP have different foraging strategies depending on the colony location, they were sampled in two contrasting marine environments, an enclosed bay (Penn Island, located in the large Baie du Morbihan) and an open water site (Estacade) (Table 1).
Penguin moult involves two distinct processes, with new feather synthesis and old feather loss overlapping in mid-moult (Cherel et al., 1994). Thus both new and old feathers from the same individual adult penguins were 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 molts that occurred during the 2005–2006 and 2006–2007 austral summers (hereafter called 2006 and 2007), respectively. Feathers (2007 moult) were also sampled from chicks at fledging (i.e. at the end of the breeding season) and, for KP only, from moulting immature birds (i.e. young birds after their first year at sea). Between 6 and 10 body feathers per individual were pulled out and then 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 using a 2:1 chloroform:methanol solution followed by two successive methanol rinses. After cleaning, body feathers were oven dried for 48 hours at 50°C. In a first step, an individual feather per penguin was analysed for total Hg in an Advanced Mercury Analyzer spectrophotometer (Altec AMA 254) following Blévin et al. (2013). Since almost all Hg is under organic form in feathers, total Hg approximates the amount of feather methyl-Hg (Bond and Diamond, 2009; Thompson and Furness, 1989). All analyses were repeated 2-3 times until having a relative standard deviation < 10%. Accuracy was checked using a certified reference material (Tort-2 Lobster Hepatopancreas, NRC, Canada; certified Hg concentration: 0.27 ± 0.06 µg·g⁻¹ dry mass). Our measured values were 0.226 ± 0.003 µg·g⁻¹ dry mass, n = 7. Blanks were analysed
at the beginning of each set of samples and the detection limit of the method was 0.005 µg·g⁻¹ dry mass. Data of Hg concentrations are presented relative to the dry weight (dw). In a second step, an individual feather per adult 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 analyzer (Thermo Scientific Flash EA 1112). Results are presented in the usual δ notation relative to Vienna PeeDee 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.

2.3. Statistical analysis

Statistical tests were performed using R 2.7.1 (R Development Core Team, 2008) mainly following Crawley (2007). In univariate analysis, all 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 and followed by multiple comparisons tests. Relationships between feather Hg concentrations and continuous explanatory variables (feather δ¹³C and δ¹⁵N values) were tested using Pearson or Spearman correlation rank tests. Multivariate analyses were used to test multiple alternative hypotheses on the influence of species, foraging habitat and trophic level (inferred from feather δ¹³C and δ¹⁵N values, respectively) on feather Hg concentrations. Generalized linear models (GLM) with a normal distribution and an identity-link function were constructed as follows: log-transformed Hg concentrations as the response variable, species as a categorical
explanatory variable and feather $\delta^{13}C$ and $\delta^{15}N$ values as continuous explanatory variables. Biologically relevant models were constructed incorporating the different variables and their interactions. Continuous variables ($\delta^{13}C$ and $\delta^{15}N$) that were significantly correlated were not included in the same models. Model selection was based on Akaike’s Information Criteria adjusted for small sample sizes (AIC$_c$). The model with the lowest AIC$_c$ value was considered to be the most accurate. Models with AIC$_c$ values differing by less than 2 have a similar level of support in the data, and the model including the least number of parameters was regarded as the most accurate, according to the principle of parsimony (Burnham and Anderson, 2002). Overall model support was assessed using Akaike weights ($w_i$), following Johnson and Omland (2004) and model fit was checked by residual analysis. A significance level of $\alpha < 0.05$ was used for all tests, both in univariate and multivariate analysis. Values are means ± SD.

3. Results

3.1. Influence of species and foraging ecology on Hg concentrations in adult penguins

In a first step, univariate analyses were used to test interspecific differences in feather Hg concentrations and stable isotopes signatures and their relationships in adult Kerguelen penguins. Feather Hg concentrations of adult penguins were significantly different between species in 2007 (Kruskal-Wallis, $H = 17.6$, $p = 0.001$, $n = 48$). KP, MP and SRP presented similar feather Hg levels, whereas GP were significantly more contaminated and showed a much higher variance (Table 2). Feather $\delta^{13}C$ and $\delta^{15}N$ values varied significantly within the penguin community (Kruskal-Wallis, $H = 35.5$ and $H = 36.5$ for $\delta^{13}C$ and $\delta^{15}N$, respectively, both $p < 0.0001$, $n = 48$), defining three
non-overlapping isotopic niches, with identical isotopic signatures of the closely-related MP and SRP (Table 2).

When individual data from the four penguin species were pooled (data not shown), feather Hg concentrations were significantly and positively correlated to both feather δ¹³C and δ¹⁵N values (Spearman correlation, ρ = 0.42 and 0.50, p = 0.003 and 0.0003, respectively, both n = 48). Feather δ¹³C and δ¹⁵N values were also significantly and positively correlated (Pearson correlation, r = 0.79, p < 0.0001, n = 48).

In a second step, multivariate analyses were used to disentangle the influence of species, foraging habitat (δ¹³C) and trophic level (δ¹⁵N) on feather Hg concentrations of adult penguins. Since feather δ¹³C and δ¹⁵N values were correlated, models including both variables were not included in the set of candidate models (Table 3). Models including feather δ¹⁵N values as covariate presented a better fit to the data than those including feather δ¹³C values. Two models, Log Hg = δ¹⁵N and Log Hg = δ¹⁵N + species, had a similar level of support in the data, the most accurate being Log Hg = δ¹⁵N according to the principle of parsimony.

3.2. Influence of year and age-class on feather Hg concentrations

Feather Hg concentrations in adult penguins were not significantly different between the two consecutive years, with every species exhibiting similar levels in 2006 and 2007 (Fig. 1). Interestingly, feather Hg concentrations were highly and positively correlated between the two years at the individual level (pooled data from the four species) (Pearson correlation, r = 0.89, p < 0.0001, n = 48, Fig. 2).

Chicks showed significantly lower feather Hg concentrations than adults for all the four species (Fig. 1). Moreover, KP chicks were significantly less contaminated than immature birds (pairwise Wilcoxon comparisons with Bonferroni correction, p = 0.005,
While feather Hg concentrations of immature and mature birds were not significantly different (pairwise Wilcoxon comparisons with Bonferroni correction, $p = 0.220$, $n = 36$; Table 2). Finally, chick feather Hg concentrations differed significantly between species (Kruskal-Wallis, $H = 41.16$, $p < 0.0001$, $n = 48$), in the decreasing order GP > KP > MP > SRP (Table 2).

3.3. Spatial and individual variation of feather Hg concentrations: the case study of adult GP

In 2007, feather Hg concentrations of adult GP differed according to the colony of origin, with individuals breeding in an enclosed bay (Penn Island) being significantly less contaminated than those breeding at an open-sea location (Estacade) (Wilcoxon, $W = 137$, $p < 0.0001$, $n = 24$). Unlike Penn GP, Estacade birds showed a high variance in their feather Hg concentrations, and also in their $\delta^{13}C$ and $\delta^{15}N$ values (Table 2). Interestingly, feather Hg concentrations from Estacade penguins were significantly and positively related to both their $\delta^{13}C$ and $\delta^{15}N$ values (Pearson correlation, $r = 0.61$, $p = 0.037$ and $r = 0.74$, $p = 0.006$, respectively, $n = 12$) (Fig. 3).

4. Discussion

4.1. Adult Hg levels: comparison with other seabirds and other areas

The Kerguelen penguin community presented two groups of Hg contamination: on the one hand KP, MP and SRP with similar feather Hg concentrations and on the other hand GP, with ~3 fold higher levels than the three other species. Based on multivariate analysis, the effect of species explained a considerable proportion of the variance in feather Hg levels, as previously shown in other subantarctic seabird communities
Furthermore, the interspecific differences were consistent between years. Indeed, no inter-annual variation was detected in feather Hg concentrations between 2006 and 2007 both at the species and individual levels. This result is consistent with the previous findings of Scheifler et al. (2005) on adult KP from the Crozet Islands (southern Indian Ocean), which showed no variation in feather Hg concentrations between 2000 and 2001.

Overall, feather Hg concentrations of Kerguelen penguins were in the same order of magnitude as those reported by previous studies on penguins, but discrepancies at the species level exist. While feather Hg concentrations of KP from Crozet (Scheifler et al., 2005) were almost identical to those found in this study, GP at South Georgia (southern Atlantic Ocean) presented lower feather Hg levels (Becker et al., 2002) than their Kerguelen conspecifics. Interestingly, South Georgia GP presented lower feather Hg levels than the sympatrically breeding MP, which is opposite to the findings of the present study. These contrasting results within the same species breeding at distinct sites are likely related to different feeding habits. Indeed, diet composition of a given species can vary according to breeding location (e.g., Lescroel et al., 2004; see Section 4.3.), and diet is the main factor explaining Hg burden in seabirds (e.g., Bocher et al., 2003; Monteiro et al., 1998; Stewart et al., 1999).

Feather Hg concentrations of penguins rank low to intermediate when compared to most procellariiform seabirds that breed in the Southern Ocean. For instance, penguins are usually more contaminated than small zooplankton-eating petrels, but are less contaminated than sympatrically breeding albatrosses and large petrels, which generally present feather levels > 6-10 µg·g⁻¹ (Anderson et al., 2009; Bargagli et al., 1998; Becker et al., 2002; Lock et al., 1992). Finally, penguins present lower feather Hg concentrations than other diving seabirds from the Southern Ocean, like the South
Georgian shag and the common and South Georgian diving petrels (Anderson et al., 2009; Becker et al., 2002). These Hg differences between penguins and other seabirds can be related more to intrinsic (e.g. physiology) and extrinsic factors (e.g. diet composition) than to phylogeny (Anderson et al., 2009; Stewart et al., 1999).

4.2. Chick Hg levels and age-class variation

As expected, feather Hg concentrations of adults were significantly higher than those of chicks in all Kerguelen penguin species. The same result has been reported by several other studies on birds (e.g., Bond and Diamond, 2009; Burger, 1993; Thompson et al., 1991), including penguins (Bargagli et al., 1998), the most likely explanation being that adults have a longer period to bioaccumulate Hg than chicks. Indeed, feather Hg levels in chicks only represent exposure during the chick-rearing period, as the Hg burden inherited from the mother via the egg is excreted, at least partially, in the down (Bearhop et al., 2000a; Becker et al., 1993; Stewart et al., 1997) and is highly diluted in the internal tissues during growth (Ackerman et al., 2011). As the length of the chick-rearing period varies among penguins (for review, Williams, 1995), the ratio of adult to chick feather Hg might be expected to be higher in species with shorter chick-rearing periods (Furness et al., 1990). In the present study, the ratios were as high as ~6 and ~7 in MP and SRP, respectively, having chick-rearing periods of ~70 days (for review, Williams, 1995). In contrast, the ratio was only ~2 in KP, because their chick-rearing period is long (~315 days; Williams, 1995) and close to the adult inter-moult period (~365 days). Accordingly, feather Hg concentrations of immature KP were similar to those of adults, in agreement with the identical duration of their inter-moult periods. A remarkable exception was the GP with an adult to chick feather Hg ratio of only ~2, even if their chick-rearing period is short (72 days on average; Williams, 1995). The
time of exposure is thus not the only determinant of feather Hg concentrations, but other intrinsic or extrinsic factors are likely involved. For instance, the small difference in contamination between the two GP age classes might be linked to the fact that the diet of adults was less Hg-enriched than that of the chicks. Indeed, seabirds can present parent-offspring dietary segregation, as adults tend to provision their chicks with larger and more energy-rich prey than those they capture for self-feeding (Alonso et al., 2012; Dänhardt et al., 2011; Wilson et al., 2004).

Interestingly, interspecific differences were not identical when considering either adults or chicks. With the exception of GP, showing the highest feather Hg concentrations in both age classes, the other species presented feather Hg concentrations in the order KP>MP>SRP in chicks while they were similar in adults. Two non-exclusive factors can explain this result: the length of the chick-rearing period and the chick diet. Indeed, the long chick rearing duration of KP could account for its high chick feather Hg level with respect to MP and SRP. Moreover, the main food items of KP chicks at different locations are mesopelagic fish (e.g., Bost et al., 2002; Cherel et al., 1996), which are known to accumulate high Hg burdens (Bustamante et al., 2003; Chouvelon et al., 2012; Monteiro et al., 1998) in relation to elevated Hg methylation in low oxygen, mesopelagic waters (for review, see Fitzgerald et al., 2007). By contrast, MP and SRP chicks have a mixed diet, relying mostly on swarming pelagic crustaceans (Table 1), which have lower Hg concentrations than fish (e.g., Anderson et al., 2009). This pattern is in agreement with the findings of Blévin et al. (2013) for Hg concentrations in Kerguelen seabird chick feathers, which increased roughly in the order crustacean-<fish-≤squid-≤seabird-consumers. Their data ranked the penguin species in a low (KP, MP, SRP) to intermediate (GP) position within the seabird community. As penguins are not the most contaminated family among Antarctic and subantarctic seabirds, it could be
argued that they are not the best sentinel species of Hg contamination in the Southern Ocean. However, penguins can be regarded as reliable Hg indicators over highly-mobile, flying species when considering their moult strategies and their feeding ecology.

4.3. Hg contamination and penguin food and feeding ecology

4.3.1. Trophic variation of feather Hg concentrations in adult penguins

Stable isotopes segregated the penguin community into three trophic niches: two different niches for KP and GP and an overlapping niche for MP and SRP. As stated above, two groups were discriminated according to the Hg levels: on the one hand KP, MP and SRP and on the other GP. This is in agreement with several studies showing weak or no association between stable isotopes and Hg levels in seabird feathers (Bond and Diamond, 2009; Ramos et al., 2009; Thompson et al., 1998). Indeed, feathers reflect the isotopic composition of the bird’s diet at the time of their synthesis (Kelly, 2000), while feather Hg levels reflect dietary exposure during feather growth but also Hg stored in soft tissues during the inter-moult period. Stable isotopes and Hg integration are therefore temporally uncoupled in adult bird feathers (Bond and Diamond, 2009; Bond, 2010; Thompson et al., 1998). Nevertheless, individual foraging preferences are maintained over extended periods in penguins, with respect to both foraging area and diet, during and outside the breeding season (Bost et al., 2009; Cherel et al., 2007; Thiebot et al., 2012, 2011a, 2011b). Unlike other seabirds, the relationship between isotopic ratios and Hg concentrations is therefore not spurious in adult penguin feathers. In the present study, both δ^{13}C and δ^{15}N values were correlated to feather Hg concentrations. With respect to the foraging habitat (δ^{13}C values), the high feather Hg concentrations in feathers of GP, the only inshore feeder, is in agreement with the Me-Hg enrichment of coastal benthic areas (Fitzgerald et al., 2007). Nevertheless,
multivariate analyses revealed that the trophic position (δ^{15}N values) had a stronger effect on feather Hg concentrations than the foraging habitat. This result verifies our hypothesis stating that Hg concentration increases with trophic level, thus indicating Hg biomagnification in the marine ecosystem exploited by the penguins, i.e. Kerguelen neritic and oceanic waters.

4.3.2. Spatial and individual variation of feather Hg concentrations: the case study of adult GP

While inter-specific differences in seabird Hg contamination have been extensively studied, the causes underlying variation within the same species are less well understood (Bearhop et al., 2000b). However, intra-specific variations in seabird Hg concentrations potentially reflect the effect of interacting intrinsic factors like age, sex and size (Burger, 1993; Monteiro and Furness, 1995) and extrinsic factors, such as foraging habitat and diet (Bearhop et al., 2000a, 2000b; Ramos et al., 2009; Stewart et al., 1997). In this study, the two sampled sub-populations of GP showed highly different levels of Hg contamination, with Estacade GP displaying on average 4 times higher feather Hg concentrations than their Penn Island conspecifics. Such striking differences in feather Hg concentrations within the same species seem difficult to attribute to physiological factors. Instead, Kerguelen GP show a great variability in their trophic ecology depending on the colony location (Bost and Jouventin, 1990; Lesroël and Bost, 2005; Lescroel et al., 2004). For instance, GP from the enclosed bay feed extensively on swarming crustaceans (85% of the diet by mass at different sites of the bay; Lescroel et al., 2004). Accordingly, they presented low feather Hg concentrations, as observed in species relying mainly on pelagic crustaceans (Becker et al., 2002; Bocher et al., 2003; Stewart et al., 1999). On the other hand, Kerguelen GP breeding at the open-sea location
Estacade present a more diversified diet, including a large proportion of benthic fish, but also pelagic crustaceans (71% and 13% of the overall diet by mass, respectively; Lescroel et al., 2004). Benthic organisms are in close association with the sediment where Hg methylation is high due to low oxygen concentrations and low solar radiation (Fitzgerald et al., 2007). They consequently tend to accumulate high levels of Hg (Bustamante et al., 2006; Storelli et al., 2005; 2007), most probably explaining the considerable Hg burden of Estacade GP. Furthermore, fish display higher Hg concentrations than crustaceans (Chouvelon et al. 2012) and virtually all the Hg (i.e. > 95%) in fish is methylated (Bloom 1992) and is therefore bioavailable for upper trophic levels. The different Hg contamination of the two colonies seems therefore to rely mainly on diet preferences.

The pattern of Hg contamination in the two colonies differed also when considering inter-individual variation. Indeed, Penn and Estacade GP showed a small and a large variance in feather Hg concentrations, respectively. Moreover, there was an overlap in feather Hg concentrations between the two colonies, with some individuals of Estacade GP showing similar levels of Penn GP and then a continuous increasing of the Hg concentration. Importantly, feather Hg levels of Estacade GP were significantly and positively correlated to stable isotopes (Fig. 3). This indicates a succession of specialised foraging individuals among Estacade GP, ranging from birds feeding almost exclusively on pelagic crustaceans (reflected by low feather $\delta^{13}$C and $\delta^{15}$N values) to birds feeding almost exclusively on benthic fish (high feather $\delta^{13}$C and $\delta^{15}$N values), therefore showing low to high feather Hg concentrations. Individual dietary specialisation is thus crucial in establishing Hg contamination in this species.
5. Conclusions

This work provides new insights into the relationship between seabird trophic ecology and Hg burdens, emphasising the role of species and individual foraging specialisation in shaping feather Hg levels. Individual specialisation of foraging strategies is believed to be conserved over long periods in seabirds (Bearhop et al., 2006; Ceia et al., 2012) and could therefore put some individuals at a risk of high Hg contamination levels over the long-term, as illustrated by the positive inter-annual correlation in feather Hg levels. Although it is difficult to link observed Hg tissue concentrations to negative effects in natural bird populations (Burger and Gochfeld, 2004), 50 % of adult Estacade GP exceeded the commonest used feather toxicity threshold of 5 µg g\(^{-1}\) dw (i.e. Burger and Gochfeld, 1997). This sub-population of GP could therefore serve as a model to investigate adverse effects of Hg contamination on seabirds in the wild. On the other hand, penguin species showing low inter-individual variation in Hg levels could be used as efficient indicators of Hg contamination. Considering their abundance, distribution and highly specialised diet (mesopelagic fish), KP in particular could be useful as a bioindicator species of Hg bioavailability in the Southern Ocean. A comparison of the results of this study with those obtained from other penguin populations which breed at different subantarctic and Antarctic sites could help to depict the geographical distribution of Hg contamination in the Southern Ocean. Furthermore, penguins could be useful as monitors of temporal trends of global Hg contamination, as their foraging strategies are maintained over the long-term. A change in Hg bioavailability in the food webs of the Kerguelen Islands, which are far-removed from direct anthropogenic inputs, would indeed indicate a change of Hg fluxes on a much wider spatial scale.
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Caption to figures:

**Fig. 1.** Feather Hg concentrations (µg·g⁻¹ dw) of penguin chicks and adults in 2006 and 2007 at Kerguelen Islands. * Statistically different (Wilcoxon, p < 0.05); NS: not significant (Wilcoxon test for matched samples, p > 0.05). Values are means ± SD.

**Fig. 2.** Positive correlation between feather Hg concentrations (µg·g⁻¹ dw) of individual adult penguins during two consecutive years (2006 and 2007) at Kerguelen Islands.

**Fig. 3.** Positive correlations between new feather Hg concentrations (µg·g⁻¹ dw) and δ¹³C and δ¹⁵N values (‰) of individual adult gentoo penguins from the open-water site (Estacade) at Kerguelen Islands.
Table 1. Foraging ecology of penguins during the breeding and non-breeding periods at the Kerguelen Islands.

<table>
<thead>
<tr>
<th>Species</th>
<th>Colony location</th>
<th>Foraging habitat</th>
<th>Chick diet</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>King penguin</td>
<td>Ratmanoff (open sea)</td>
<td>Polar Frontal Zone (oceanic; epi-mesopelagic)</td>
<td>Pelagic fish</td>
<td>Bost et al., 2002; Cherel et al., 2010.</td>
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<tr>
<td></td>
<td></td>
<td>Unknown (likely in cold oceanic waters; epi-mesopelagic)</td>
<td></td>
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<tr>
<td>Macaroni penguin</td>
<td>Cap Cotter</td>
<td>Eastwards off Kerguelen in the Polar Frontal Zone (oceanic; epipelagic)</td>
<td>Pelagic crustaceans and fish</td>
<td>Cherel et al., 2010; Thiebot et al. (2011a, 2011b).</td>
</tr>
<tr>
<td>Southern rockhopper penguin</td>
<td>Mayes, Morbihan Bay</td>
<td>Morbihan Bay (neritic; pelagic and benthic)</td>
<td>Pelagic crustaceans and fish</td>
<td>Tremblay and Cherel (2000, 2003); Cherel et al. (2010); Thiebot et al. (2012).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Subantarctic and Polar Frontal Zones (oceanic; epipelagic)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentoo penguin</td>
<td>Ratmanoff (open sea)*</td>
<td>Eastwards off Kerguelen (neritic; benthic and pelagic); Resident all year long</td>
<td>Benthic fish and pelagic crustaceans</td>
<td>Lescroel et al. (2004); Lescroël and Bost (2005).</td>
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<tr>
<td></td>
<td>Penn Island, Morbihan Bay (closed sea)</td>
<td>Morbihan Bay (coastal; pelagic)</td>
<td>Resident all year long</td>
<td>Lescroel et al. (2004); Lescroël and Bost (2005).</td>
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</tbody>
</table>

* Cape Ratmanoff is close to Cape Estacade and diet of gentoo penguins is similar in both sites.
Table 2. Feather Hg concentrations (µg·g⁻¹ dw) and δ¹³C and δ¹⁵N values (‰) of Kerguelen penguins in 2006 and 2007. Values are means ± SD (with ranges in parentheses for Hg).

<table>
<thead>
<tr>
<th>Species</th>
<th>Feather</th>
<th>Year</th>
<th>n</th>
<th>Hg a</th>
<th>δ¹³C a</th>
<th>δ¹⁵N a</th>
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<tbody>
<tr>
<td>King penguin</td>
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<td></td>
</tr>
<tr>
<td>Adults</td>
<td>New</td>
<td>2007</td>
<td>12</td>
<td>2.22</td>
<td>-21.29</td>
<td>10.90</td>
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<tr>
<td>Adults</td>
<td>Old</td>
<td>2006</td>
<td>12</td>
<td>2.17</td>
<td>1.45-3.21</td>
<td>1.22-3.14</td>
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<tr>
<td>Immatures</td>
<td>New</td>
<td>2007</td>
<td>12</td>
<td>1.79</td>
<td>1.93-2.77</td>
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<tr>
<td>Chicks</td>
<td>New</td>
<td>2007</td>
<td>12</td>
<td>1.12</td>
<td>0.83-1.50</td>
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<tr>
<td>Adults</td>
<td>New</td>
<td>2007</td>
<td>12</td>
<td>2.24</td>
<td>1.87-2.75</td>
<td>20.07</td>
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<td>Adults</td>
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<td>2006</td>
<td>12</td>
<td>2.08</td>
<td>1.64-2.90</td>
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<tr>
<td>Chicks</td>
<td>New</td>
<td>2007</td>
<td>12</td>
<td>0.36</td>
<td>0.25-0.52</td>
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<tr>
<td>Southern rockhopper penguin</td>
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<tr>
<td>Adults</td>
<td>New</td>
<td>2007</td>
<td>12</td>
<td>1.96</td>
<td>1.22-2.62</td>
<td>19.90</td>
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<td>2006</td>
<td>12</td>
<td>1.92</td>
<td>1.30-2.49</td>
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<td>Chicks</td>
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<td>2007</td>
<td>12</td>
<td>0.27</td>
<td>0.20-0.37</td>
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<td>Gentoo penguin</td>
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<tr>
<td>Estacade</td>
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<tr>
<td>Adults</td>
<td>New</td>
<td>2007</td>
<td>12</td>
<td>5.85</td>
<td>1.28-9.43</td>
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<td>2006</td>
<td>12</td>
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<td>2007</td>
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<td>2.45</td>
<td>1.14-3.66</td>
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<td>Penn Island</td>
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<tr>
<td>Adults</td>
<td>New</td>
<td>2007</td>
<td>12</td>
<td>1.44</td>
<td>0.77-2.06</td>
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</tbody>
</table>

*Groups with the same letter are not statistically different (pairwise Wilcoxon comparisons with Bonferroni correction, p < 0.05). Upper and lower-case letters are for adults and chicks, respectively.*
Table 3. AIC<sub>c</sub> model ranking for adult feather Hg concentrations within the Kerguelen penguin community. Abbreviations: AIC<sub>c</sub>, Akaike’s Information Criteria adjusted for small sample-sizes values; w<sub>i</sub>, AIC<sub>c</sub> weights; R<sup>2</sup>adj, R-squared adjusted.

<table>
<thead>
<tr>
<th>Models</th>
<th>N° parameters</th>
<th>AIC&lt;sub&gt;c&lt;/sub&gt;</th>
<th>ΔAIC&lt;sub&gt;c&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</th>
<th>w&lt;sub&gt;i&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;adj</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ&lt;sup&gt;15&lt;/sup&gt;N</td>
<td>3</td>
<td>26.30</td>
<td>0.00</td>
<td>0.54</td>
<td>0.67</td>
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<tr>
<td>species + δ&lt;sup&gt;15&lt;/sup&gt;N</td>
<td>6</td>
<td>26.68</td>
<td>0.38</td>
<td>0.44</td>
<td>0.69</td>
</tr>
<tr>
<td>species + δ&lt;sup&gt;15&lt;/sup&gt;N + species:δ&lt;sup&gt;15&lt;/sup&gt;N</td>
<td>9</td>
<td>32.72</td>
<td>6.41</td>
<td>0.02</td>
<td>0.69</td>
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<tr>
<td>species + δ&lt;sup&gt;13&lt;/sup&gt;C + species:δ&lt;sup&gt;13&lt;/sup&gt;C</td>
<td>9</td>
<td>40.08</td>
<td>13.78</td>
<td>0.00</td>
<td>0.64</td>
</tr>
<tr>
<td>species + δ&lt;sup&gt;13&lt;/sup&gt;C</td>
<td>6</td>
<td>40.10</td>
<td>13.79</td>
<td>0.00</td>
<td>0.59</td>
</tr>
<tr>
<td>δ&lt;sup&gt;13&lt;/sup&gt;C</td>
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<td>41.24</td>
<td>14.94</td>
<td>0.00</td>
<td>0.55</td>
</tr>
<tr>
<td>Species</td>
<td>5</td>
<td>49.20</td>
<td>22.89</td>
<td>0.00</td>
<td>0.49</td>
</tr>
<tr>
<td>Null</td>
<td>2</td>
<td>77.85</td>
<td>51.54</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

<sup>a</sup> Scaled ΔAIC<sub>c</sub>; ΔAIC<sub>c</sub> = 0.00 is interpreted as the best fit to the data among the models

<sup>b</sup> Weight of evidence interpreted as a proportion. Weights across all models sum to 1.00
Fig 1.
Fig 2.
Fig 3.

Pearson correlation

- $p$ value = 0.037
- $r$ = 0.61

Gentoo penguins
(Estacade)

Pearson correlation

- $p$ value = 0.006
- $r$ = 0.74