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Mercury accumulation in Gentoo penguins *Pygoscelis papua*: spatial, temporal, and intraspecific variations

Sara Joana Pereira Pedro

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Dissertation presented to Universidade de Coimbra in fulfillment of the requirements needed to obtain the Master degree in Ecologia, accomplished under the scientific supervision of Doctor Miguel Pardal (Universidade de Coimbra) and Doctor José Xavier (Universidade de Coimbra).

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Mercury accumulation in Gentoo penguins *Pygoscelis papua*: spatial, temporal, and intraspecific variations

RESUMO

As emissões de mercúrio aumentaram nas últimas décadas, afetando áreas remotas, como a Antártida. A localização das colónias de pinguim-gentoo (*Pygoscelis papua*) na região da Antártida faz desta espécie um bom bio-monitor das concentrações de mercúrio nesta região. Este trabalho avalia as concentrações de mercúrio nas penas de pinguim-gentoo em Bird Island, Geórgia do Sul nos anos de 2009 e 2010. A localização desta ilha mais próximo da ação humana pode significar um maior nível de contaminação, numa das maiores colónias desta espécie. Além disso, o conhecimento prévio das interações tróficas neste local permite a compreensão da transferência de mercúrio ao longo da cadeia trófica. Não houve diferenças significativas nos níveis de mercúrio entre 2009 e 2010 (0.97±0.67 mg kg^{-1}; 1.13±0.62 mg kg^{-1}, respetivamente). Verificou-se um aumento dos níveis de mercúrio com o aumento do peso e da proporção de peixe na dieta dos pinguins. No entanto, o género não teve influência nestes níveis. Os coeficientes de variação foram altos em 25% da população, a qual apresentou diferenças significativas entre os valores máximos e mínimos de concentração de mercúrio nas penas. Esta variação pode ser causada por diferenças nos padrões de muda entre indivíduos da mesma população, o que pode levar a diferentes interpretações quando toda a população é analisada. Não foram verificadas variações temporais nos níveis de mercúrio nas penas destes pinguins, mas, em comparação com estudos anteriores, as diferenças espaciais na região Antártida são evidentes. Estes resultados demonstram que os animais de nível trófico superior, cuja alimentação se baseie nesta espécie, estão sujeitos a potenciais efeitos no seu desenvolvimento e reprodução.

Palavras-chave: mercúrio; nível trófico; pinguim; Antártida.

ABSTRACT

Mercury emissions have increased over the last decades and are affecting even remote areas such as Antarctica. As Gentoo penguins (*Pygoscelis papua*) breed around Antarctica and forage nearby their colonies, they may characterize the mercury concentrations in the region. We evaluated here the mercury concentrations in Gentoo penguin feathers at Bird Island, South Georgia at 2009 and 2010. Its location close to anthropogenic sources may indicate a higher level of contamination in one of the largest colonies of this species. Besides, trophic interactions here are well-studied, allowing the understanding of mercury transference through food-webs. There were no significant differences in mercury levels between 2009 and 2010 (0.97±0.67 mg kg^{-1}; 1.13±0.62 mg kg^{-1}, respectively). There was an increase of mercury levels with increasing weight and proportion of fish on penguins' diet, but sex had no influence on it. The coefficients of variation were high in 25% of the population which presented significant differences between the minimum and maximum value of mercury concentrations in the feathers. This may be caused by differences in moult patterns within the population leading to different interpretations if included in the overall population. No temporal variations in levels of mercury in Gentoo penguin feathers were observed, but spatial differences are clear in the Antarctic region when comparing with previous studies elsewhere around Antarctica. These results highlight possible implications for other animals up in the food web that feed considerably on gentoo penguins, namely on reproduction and development.

Keywords: mercury; trophic level; penguin; Antarctica.

CONTENTS

1. INTRODUCTION

The Antarctic continent, located in the Southern Hemisphere and containing the South Pole (magnetic and geographic), was separated from the other continents about 30 Ma ago. Atmospheric and oceanic circulation, namely the Antarctic Circumpolar Current (ACC), contribute to its remoteness, preventing the entrance of air masses and water from lower latitudes, and thus allowing the maintenance of Antarctica's ice sheet (Bargagli, 2008). For this reason, the Antarctic is generally considered to be a pristine area, where existing contamination levels are low. However, as with other remote regions on the planet, it is impacted by local and global anthropogenic activities (Bargagli, 2008; Streets et al., 2009). Over the last three decades global pollution, such as chlorofluorocarbons (CFC), CO_2, mercury, pesticides and other Persistent Organic Pollutants (POPs) have been detected in this region (Bargagli, 2008). Anthropogenic activities change atmosphere-ocean heat fluxes and oceanic circulation leading to temperature increases in the Southern Ocean (Whitehouse et al., 2008). Local anthropogenic activities are also directly affecting the Antarctic biodiversity (Campos et al., 2013). The causes that have been pointed out in this matter are the increase of tourism activities in the region, fisheries and research (Bargagli, 2008; Moreno et al., 1997). Also, the accidental input of invasive species in Antarctica can spread and cause severe impacts by competing with native species (Chown et al., 2012).

Mercury (Hg) is one of the most concerning contaminants on the marine environment (Bargagli, 2008). It is a non-essential metal, once dissolved in water become bioavailable, and toxic when its concentrations are elevated above certain levels (Boening, 2000; Booth and Zeller, 2005). Although there are natural sources of mercury (eg. volcanoes, geothermal activity, weathering of mercury-containing rocks), anthropogenic activities have increased largely its release to the environment. In fact, it was estimated that over the last 100 years, emissions from anthropogenic activities have doubled the concentrations of mercury in the surface of the oceans (UNEP, 2013). These activities include the burning of fossil fuels, mining, chloralkali industry and incineration of

products containing mercury, such as batteries, paints, electrical and electronic devices, fluorescent and energy-saving lamps, blood-pressure gauges, medicines and thermometers (Boening, 2000; UNEP, 2013).

Mercury can be found in different chemical forms in the environment. When it is in the elemental form, mercury can persist in the atmosphere for a long time and thus can be transported around the globe. When oxidized or in a particulate form, mercury can be transported to land and oceans (Barkay and Poulain, 2007; UNEP, 2013). During its cycle, this contaminant can be transformed and transported indefinitely among soil, water and air (UNEP, 2013).

Atmospheric deposition into the aquatic environment holds the three most important forms of mercury: elemental mercury (Hg^0), inorganic mercury (Hg^{2+}, mercury salts) and the most common form of organic mercury (methylmercury) (CH_3Hg). These three forms can be converted to each other by methylation, demethylation and reduction. The most common form in water is inorganic mercury, which can be methylated into organic mercury (methylmercury) by natural bacterial activity, mainly on the subsurface of the water (Barkay and Poulain, 2007; Boening, 2000). This form of mercury can persist in the water for about 11 years and is the most toxic one, since it is absorbed by organisms easier than the inorganic form and has the capacity to bio-accumulate and bio-magnify through food webs (Brasso et al., 2012; Coelho et al., 2010; Nygård et al., 2001). Bioaccumulation is the ratio between mercury concentrations in the environment and in the organisms and biomagnification is the increase in concentrations through the food web, being higher in upper trophic levels (UNEP, 2013). This fact leads to high concentrations of mercury in top predators. The impacts of the bioaccumulation processes in organisms depend upon several factors such as species, trophic level, age, life span, sex, moult pattern and diet (Becker et al., 2002; Bocher et al., 2003; Frias et al., 2012).

When taken by organisms, methylmercury is transported through blood and lymph vascular systems to the organs where it will be deposited, demethylated or excreted (Dietz et al., 2013). The organs where mercury tends to accumulate in a higher proportion are species-dependent. It is known that animals have the capacity to demethylate mercury in the liver and store it as

inorganic molecules (Bargagli, 2008; Burger and Gochfeld, 2004). Inorganic mercury has the capacity to bind to selenium molecules, which partially protects animals from mercury adverse effects. Other strategies include excretion by binding of methylmercury to growing hair and feathers (Dietz et al., 2013).

Many toxic effects of mercury have been reported worldwide, but standard levels that cause toxicity in each species are not well-known. Mercury is primarily a neurotoxin, due to its high affinity for protein thiols (Dietz et al., 2013). One example of a severe case of mercury poisoning occurred in Minamata Bay, Japan (Harada, 1955). It was caused by the discharge of waste from a chemical plant with high levels of methylmercury in the bay. Fish species were contaminated and humans who ate these species died or suffered toxic effects. Marine products had mercury levels from 5.61 to 35.7 mg kg^{-1} and human hair showed a maximum of 705 mg kg^{-1} (Harada, 1955). Toxic effects of mercury include severe disabilities, ataxia, sensory impairment, reduced reproduction and survival rates and alteration of behaviour (Burger and Gochfeld, 1997; Dietz et al., 2013; Hallinger et al., 2011, 2010; Tan et al., 2009).

Special attention is given to marine top predators, since it has been reported that fish-eating species have higher mercury levels than non-fish eating species (Boening, 2000; Booth and Zeller, 2005).This is of concern in the Southern Ocean, where mercury levels are increasing alarmingly (Cossa et al., 2011), being reflected at the Scientific committee on Antarctic Research (SCAR) by having an expert scientists group investigating Antarctic contaminants (SCAR ECA- Environmental contamination in Antarctica). In addition, food chains in this region are very simple and depend mainly on krill and fish species (Nygård et al., 2001). Some studies demonstrate that, in the Antarctic, the accumulation of mercury in organisms shows levels on the same range as related species from other regions (Becker et al., 2002; Kojadinovic et al., 2007). Wandering albatross (*Diomedea exulans*) breeding at Bird Island, South Georgia (Southern Ocean) is the species that shows the highest mercury concentration so far reported amongst seabirds in the world (Hindell et al., 1999; Tavares et al., 2013). For this reason, there is a need to assess the levels of mercury of other seabird species in that region.

Antarctic seabirds can be an important tool to monitor environmental and animal health (Metcheva et al., 2011). One limitation on their use is that many flying seabirds range widely over the ocean and may forage well north of Antarctic waters where contamination levels are higher (Bargagli, 2008; Carravieri et al., 2013; Tavares et al., 2013). Unlike flying species, diving species such as penguins are more restricted in their use of the oceans (Carravieri et al., 2013). Gentoo penguins (*Pygoscelis papua*) are particularly constrained as they stay in their breeding colonies during the breeding season and they come ashore every day during the rest of the year (Tanton et al., 2004). They feed up to 30 km from shore, mainly on Antarctic krill *Euphausia superba* (and other crustacean species) and fish (Lescroël et al., 2004; Tanton et al., 2004). These characteristics make this species a good bio-monitor for studying mercury concentration in organisms and accumulation process in Antarctica food webs. Moreover, the Antarctic and sub-Antarctic distribution of their many colonies (Metcheva et al., 2006) allow spatial comparisons of mercury concentrations in these regions.

As all seabird species, gentoo penguin excretes mercury into feathers to reduce their body burden (Becker et al., 2002; Brasso et al., 2012). Moult is usually the major excretory pathway, releasing 60 to 93% of the birds' mercury body burden (Ancora et al., 2002; Condon and Cristol, 2009; Monteiro and Furness, 1995). This release occurs when blood mercury binds to sulfhydryl groups of keratin in the new feather during the moult. Mercury concentrations in feathers have an exclusive internal origin, reflecting the mercury dietary intake before feather growth (Bond, 2010; Ek et al., 2004; Goede and De Bruin, 1986; Jaspers et al., 2007). It is thus assumed that the first moulted feathers may have higher mercury levels than those which moult later (Dauwe et al., 2003; Head et al., 2011). Collecting feathers is easy and does not harm the birds. Moreover, feathers can be stored for very long time, allowing monitoring based on periodical moult and museum species (Monteiro and Furness, 1995; Thompson et al., 1993). Since gentoo penguins moult annually, their feathers are representative of the environmental mercury concentrations of that year (Davis et al., 1989).

Timing of moult and moult sequence are factors that depend on seabirds' lifecycle and, as a consequence, vary widely among species. In this context, it

is important to take into account differences in moult patterns that can influence mercury levels between species, individuals and even within feathers of the same individual (Bond and Diamond, 2008; Carravieri et al., 2014a; Furness et al., 1986; Head et al., 2011). Most birds have a sequential moult, starting with the replacement of the innermost primary and ending in the outermost primary (Furness et al., 1986). As a result, there is a pattern of decreasing mercury levels in primaries in these seabird species. Unlike other seabirds, penguins have a simple moult pattern, replacing all their feathers in a period of 2 to 5 weeks (Ancel et al., 2013; Carravieri et al., 2014a; Cherel, 2010; Davis et al., 1989). Gentoo penguins take approximately 4 weeks to moult and do it annually (Davis et al., 1989). Thus, it is expected and assumed by researchers that with this kind of moult all body feathers show similar levels of mercury within individuals. However, there is no evidence to support this assumption on this species.

In general, the effect of intraspecific variations in mercury accumulation is well studied in bird species. As the major internal mercury source in seabirds is food, diet is often considered the main reason for intra and interspecific differences in mercury levels (Carravieri et al., 2014b; Frias et al., 2012; Kojadinovic et al., 2007). Nevertheless many authors have proven sex and age differences, especially when comparing mercury concentration in chick and adult tissues (Becker et al., 2002; Carravieri et al., 2014b; Frias et al., 2012; Stewart et al., 1997; Tavares et al., 2013). In fact, while some studies have already reported mercury concentrations in gentoo penguin's feathers (Becker et al., 2002; Carravieri et al., 2013; Santos et al., 2006), none has focused on penguin body mass, nor on relating mercury levels with diet and moult patterns and only very few on sex variations (Becker et al., 2002).

Under such context, the aims of this study are 1) to assess the effect of sex, size and reproductive success in the mercury accumulation in feathers from gentoo penguins breeding at Bird Island, South Georgia; 2) to assess if there is variability in body feathers within-individual gentoo penguins, a species that moult all their feathers in a short period; 3) to assess the impact on this variability when interpreting the species patterns within this kind of moult species ; 4) to evaluate what is the best method for feather analysis in this kind of study; 5) to check for temporal (decadal) variations in mercury contamination

in gentoo penguins based on previous studies around Antarctica and 6) to understand if there are spatial differences or any latitudinal gradient in mercury levels from different colonies.

2. MATERIAL AND METHODS

2.1 Sampling procedures

Gentoo penguin feathers were collected on Bird Island, South Georgia (54°S 38°W) during the austral winter of 2009 (June-September) and the austral summer of 2011 (January). In 2009, for each month, 13 to 15 individuals were sampled within four-day periods. Penguins were captured at dusk as they returned from foraging and weighed using a 10kg Pesola® spring balance (Pesola AG, Barr, Switzerland). Feathers (six to eight chest feathers) were collected, following Cherel et al. (2006), Cherel and Hobson (2007) and Phillips et al. (2011).

The sex of all individuals studied in 2009 was later determined genetically: DNA from blood (samples collected at the same time as the feathers) was isolated using an adaptation of the Chelex extraction method (Walsh et al., 1991). All samples were centrifuged and a small portion of blood was removed for extraction. 50 µl of distilled H_2O and 20µl of InstaGene™ Matrix (BioRad) were added to each sample. The samples were then incubated at 50°C for 30 minutes, followed by 8 minutes at 100°C. One negative control was included for each set of 24 extractions to monitor for possible contamination with exogenous DNA.

Primers P2/P8 (Griffiths et al., 1998), commonly used for penguins (Bertellotti et al., 2002; Costantini et al., 2008), were used for PCR amplification. All PCRs included two positive controls to test for the success of the amplification and two negative controls, prepared with distilled water, to test for possible contamination. Amplifications were performed using a Multiplex kit, carried out in 10 µl reactions containing 1x of QIAGEN® Multiplex PCR Master Mix, 0.2 µM of each primer and 0.8 µl of DNA template (~1 ng μl^{-1}). The thermal

conditions were 95°C for 15 min, 35 cycles of 95ºC for 1 min, 47°C (annealing temperature) for 1 min 30 s, 72°C for 1 min 30 s, and a final extension at 72°C for 10 min. All reactions were carried out using an Applied BiosystemsVeriti® Thermal Cycler PCR machine. Samples were run for about 2h on 3% weight/volume agarose gels stained with ethidium bromide.

In 2011, the feathers were collected in a single day in January, corresponding to the moult of the year 2010 (i.e. March/April 2010). Consequently the results derived from the feathers collected in 2011 will be referenced as mercury levels during 2010.

All penguins handled in 2009 and 2011 were marked to ensure they were sampled only once, and released. The procedure lasted, on average, 15 minutes. Data on sex and weight of each individual is only available for 2009 samples.

Stomach samples were also obtained from the gentoo penguins sampled in 2009. Each food sample was collected by stomach flushing, following Xavier et al. (2004) adapted to penguins. A maximum of three flushes per sample were obtained, following the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR) Ecosystem Monitoring Program (CEMP) Standard Methods. If the first flush would provide green or yellow colour, it was assumed that they were without food and they were released immediately.

The analyses of the food samples were carried out within 24 hours of collection. Each food sample was analyzed following Xavier et al. (2003). The samples were weighed and the overall mass was recorded. To remove the liquid, each food sample was washed carefully through two sieves (1 mm and 3.35 mm). All components were then sorted into categories (crustaceans, fish, cephalopods and others; the latter comprising of debris or carrion).

2.2 Mercury quantification

By using thermal decomposition atomic absorption spectrometry with gold amalgamation in LECO AMA-254 equipment, the total mercury concentrations were measured in feathers. Due to its reduced size, the whole feather was used in mercury quantification process. Samples were weighed

directly in pre-cleaned nickel boats and its mass varied between 4.10-9.57 mg. Three or more feathers from each individual were analyzed and it was established as a maximum coefficient of variation of 10%. Solid samples placed in the nickel boats are initially dried prior to combustion inside the apparatus. The resulting mercury vapour is trapped on a gold amalgamator which then is heated to 900°C to release the mercury and transport it to a heated cuvette where it will be quantified using a silicon diode detector (Costley et al., 2000). Every day, precision and accuracy of the method were assured with the analysis of two certified reference materials: for 2009 samples it was used IAEA-407 (fish tissue; certified value = 0.22±0.006 mg kg^{-1}) with recovery efficiencies of 101.5%±6.3%, n=55; for 2010 samples it was used TORT-2 (lobster hepatopancreas certified value =0.27±0.006 mg kg^{-1}) with recovery efficiencies of 105.2% ± 1.0%, n=29. The use of reference material aims to correct daily variation and accuracy of the equipment.

2.3 Statistical analysis

In sex determination, data were statistically analyzed using Minitab statistical software (Sowers Printing Company, PA, USA). A Mann-Whitney test was used to check if there was any difference in mercury levels in feathers between 2009 and 2010. Generalized Least Squares (GLS) models were used to inspect the effect of sex, weight and diet (proportions of cephalopod, fish, crustacean and other prey) on mercury content of gentoo penguin feathers collected in 2009. All analyses were performed using the R software version 3.0.2 (R Development Core Team 2013) using the R package *nlme* (Pinheiro et al., 2013). GLS models were applied because variance in mercury concentrations increased with the linear predictors (heterocedasticity). Mercury values were log transformed and diet proportions were arcsine transformed to meet normality requirements. An information-theoretic approach was used to select the best models (Burnham and Anderson, 2002). Model selection criteria included the corrected version of Akaike's information criterion (AIC_C) for small sample sizes and the difference in AIC_C between each candidate model and the model with the lowest AIC_C value (ΔAIC_C; Burnham & Anderson 2002). The

best-fit model was selected based on the lowest AIC_C value and after graphically checking the distribution of residuals (Zuur et al., 2007).

In relation to within-individual variability, a Mann-Whitney test was used to determine if there were differences between the minimum and maximum mercury concentrations in body feathers from 2009, of each individual. Significant differences were considered when $p<0.05$. This test was performed using the software SPSS® 19.

3. RESULTS

The feathers of gentoo penguins from Bird Island showed mercury concentrations ranging between 0.15 - 3.1 mg kg^{-1} in 2009 and 0.23 - 2.5 mg kg^{-1} in 2010. There were no significant differences in the mercury concentration of gentoo penguin feathers between 2009 (0.97±0.67 mg kg^{-1}) and 2010 (mean: 1.1±0.62 mg kg^{-1}; Mann-Whitney U= 626.5, p= 0.11). In 2009, the mercury levels in birds' feathers were significantly higher in males than in females (males: 1.35±0.74 mg kg^{-1}; females: 0.68±0.42 mg kg^{-1}; GLS: t= 2.39, p= 0.02), with increasing weight (GLS: t= 2.51, p= 0.02), increasing proportion of fish (GLS: t= 2.93, p< 0.01) and in males with higher proportion of fish in their diets (GLS: t= 3.01, p< 0.01; Table I). Mercury concentrations on feathers increased significantly with an increase proportion of fish and subsequent lower proportion of crustaceans on the birds diet (Figure 1). There was some partitioning on the mercury burdens of male penguin feathers related with weight and diet. Heavyweight males feeding on a higher proportion of fish showed higher mercury concentration levels than lightweight individuals feeding on lower amounts of fish (Figure 2).

Table I: Model selection using corrected version of Akaike's Information Criterion (AIC_c) to explain mercury concentration (mg kg^{-1}) on gentoo penguin feathers as a function of the bird's (A) sex and (B) weight and (C) proportion of fish on its diet and the interaction between the former three factors. K refers to the number of parameters in each model. Bold indicates the model with the best AIC_c.

No.	Model	K	AICc	Δ AIC_c	AIC_c weight
1	**sex + weight + fish + sex * fish**	**6**	**77.83**	**0.00**	**0.69**
2	sex + weight + fish + sex * weight + sex * fish	7	79.84	2.01	0.25
3	sex + weight + fish + sex * weight + sex * fish + weight * fish	8	82.56	4.73	0.06

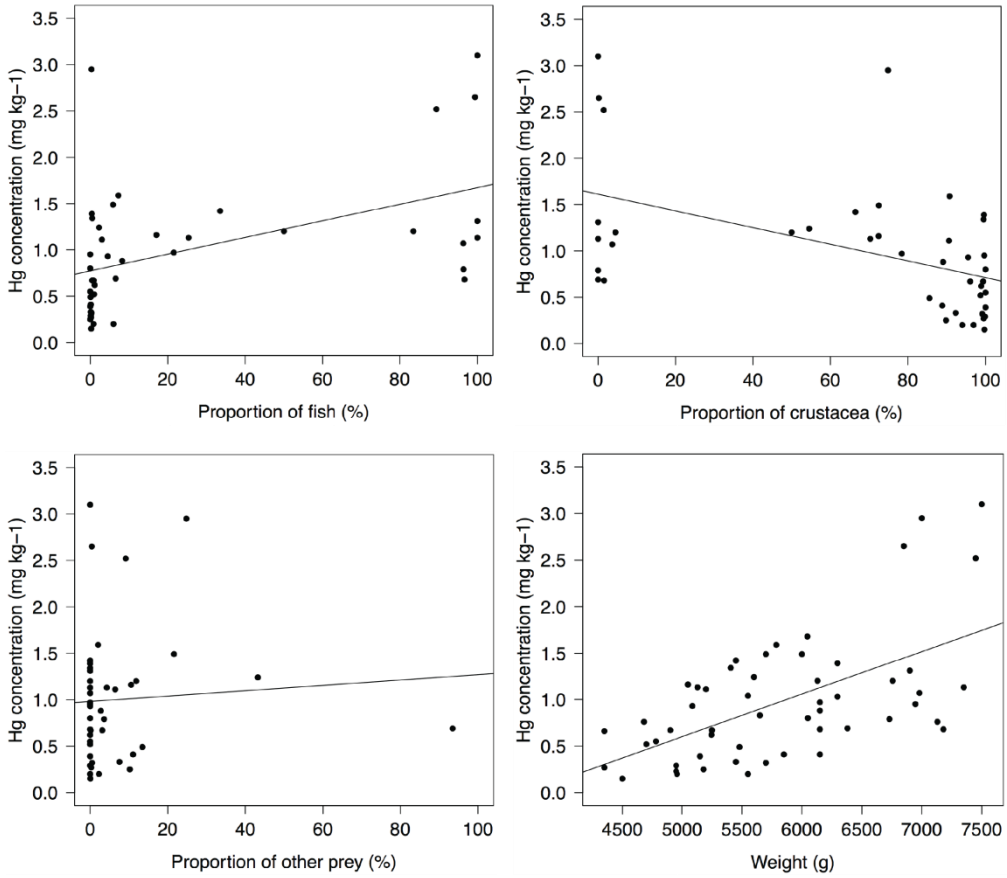

Figure 1. Relationship between mercury concentration in gentoo penguin feathers and proportions of fish, crustacean and other prey on their diet and weight of the individuals.

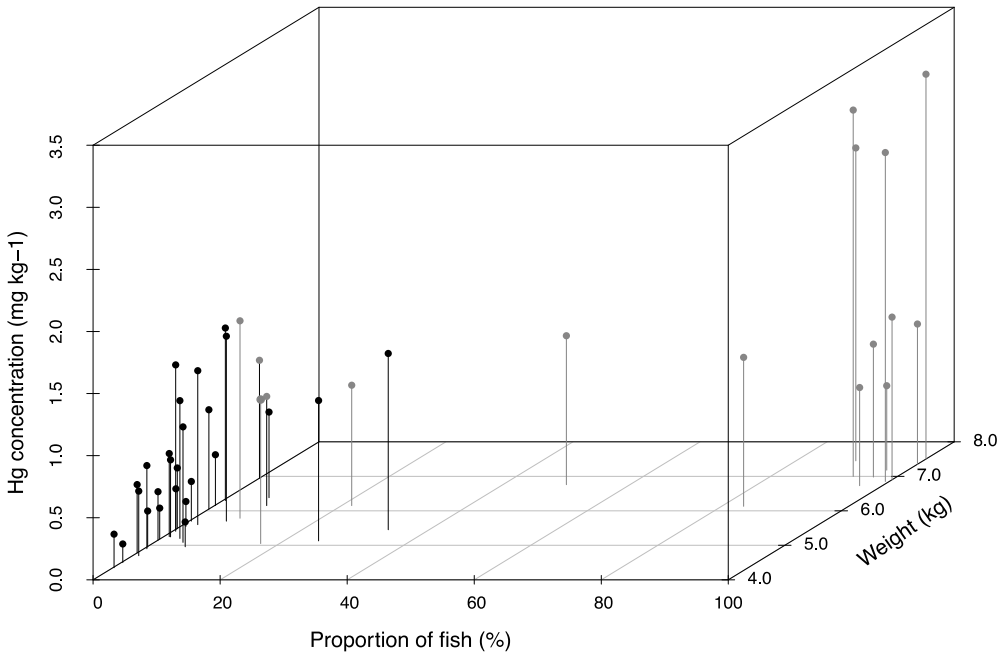

Figure 2. Mean mercury concentration (mg kg^{-1}) in gentoo penguin female (black dots) and male (grey dots) feathers, their weight and proportion (%) of fish in their diet.

There is high within-individual variability in mercury levels of gentoo penguin feathers from 2009 (Table II, Figure 3). It was assumed by the author that there is individual variation when the coefficients of variation are higher than 35% (Table II). A percentage of 25% of the population shows significant differences between the maximum and minimum mercury levels in body feathers within each individual (Mann-Whitney U= 0.00, p< 0,01). When comparing the remaining 75%, there were no significant differences (Mann-Whitney U =736.5, p =0.541, not significant). Individuals showing low coefficient of variance in feathers' mercury concentrations were considered regular and individuals with a high coefficient of variance were considered to be irregular.

Table II: Individual variation in gentoo penguins' body feathers

		Mercury (mg kg^{-1})				
Individual	**Weight (g)**	**Min**	**Max**	**Mean**	**SD**	**CV (%)**
1	6946	0,15	1,75	0,95	0,76	80
2	6296	0,43	1,93	1,03	0,72	70
3	5480	0,25	0,81	0,49	0,28	56
4	4780	0,30	0,68	0,55	0,19	35
5	5250	0,29	0,96	0,66	0,34	51
6	5050	0,58	1,44	1,16	0,42	36
7	6730	0,26	1,02	0,79	0,36	46
8	6130	0,63	2,18	1,20	0,69	57
9	6900	0,29	2,78	1,31	1,27	97
10	7350	0,42	1,88	1,13	0,76	68
11	6380	0,42	1,19	0,69	0,40	58
12	5130	0,39	1,52	1,13	0,56	50
13	6150	0,14	1,11	0,68	0,51	74

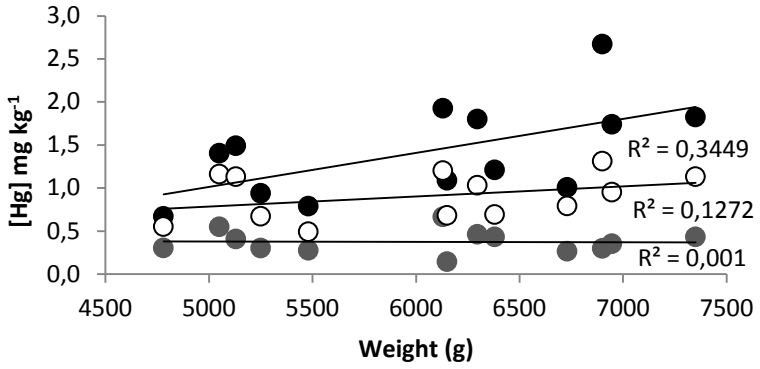

Figure 3: Different mean values for mercury concentrations in body feathers of gentoo penguins: mean of the higher concentrations (black); mean of all concentrations (white); mean of the lower concentrations (grey).

4. DISCUSSION

4.1 Intraspecific variation of mercury levels in gentoo penguins

4.1.1 Sex and weight

To our knowledge, this is the first study showing a relationship between body mass and mercury levels in gentoo penguins, with individuals with greater body mass presenting higher mercury levels in their feathers. It is known that mercury bioaccumulation increases with size in fish and seabird species

(McArthur et al., 2003; Xavier and Croxall, 2007). Lescroël and Bost (2005) concluded that gentoo penguins foraging at open sea areas tend to perform longer trips and eat larger prey, and so they tend to be bigger than those foraging at closed sea areas. As larger penguins may eat larger prey, they are expected to have greater body burdens of mercury than smaller penguins (Robinson et al., 2012). Although there is no evidence of adverse effects related to mercury in gentoo penguin populations, the results of this study may have implications for other animals through the local food web. Leopard seals (*Hydrurga leptonyx*), skuas (*Catharacta* sp.) and killer whales (*Orcinus orca*) feeding on larger gentoo penguins may bio-accumulate higher concentrations of mercury, and thus be more vulnerable to the toxic effects of this contaminant (Pitman and Ensor, 2003; Reinhardt et al., 2000; Walker et al., 1998). High concentrations of mercury are associated with alteration in behavior, reproductive and developmental effects in top predators, suppression of spawning, deficient sperm production and problems with fetus development (Hallinger et al., 2011, 2010; Tan et al., 2009; Tartu et al., 2013). In fact, clapper rails (*Rallus longirostris obsoletus*) are in risk of impaired reproduction and body mass loss with mercury levels of 9.04 mg kg^{-1} and black-legged kittiwakes (*Rissa tridactyla*) skip breeding with mercury levels higher than 2.10 mg kg^{-1} (Ackerman et al., 2012; Tartu et al., 2013).

Previous studies relating sex with mercury levels in seabirds have found no differences between males and females, e.g. macaroni penguin, magellanic penguin and some albatrosses (Becker et al., 2002; Frias et al., 2012; Stewart et al., 1999; Tavares et al., 2013). In contrast, Becker et al. (2002) found significant higher mercury levels in gentoo penguin males and related it to egg laying in females. In fact, this is considered a major elimination route for mercury in females, and it was found that gentoo penguins can release a mean of 0.2 mg kg^{-1} of mercury through the eggs (Brasso et al., 2012). In the present work, however, males and females have similar mercury levels when controlling for their mass. It is possible that sexual size dimorphism in this species may cause differences in mercury levels between males and females, as gentoo penguin males are 8% greater in body mass than females (Figure 2) (Lescroël et al., 2009). Males and females prey on the same species in the same foraging

area (Mori and Boyd, 2004), but, as males are larger than females, they may eat larger prey (Robinson et al., 2012).

4.1.2 Relationship between the diet and mercury levels of gentoo penguins

Our results suggest that the diet of gentoo penguins strongly influences the mercury levels in their feathers. This species feed mainly on crustaceans such as *Euphausia superba*, and on fish such as *Champsocephalus gunnari*, but also on cephalopods and other prey items (this study; Croxall et al. 1999; Lescroël et al. 2004). The individuals eating a higher proportion of fish and other prey items show higher mercury levels than those eating a higher proportion of crustaceans (Figure 1). These results can be explained by bio-magnification processes, as fish species generally occupy a higher position in the food chain. In fact, *Champsocephalus gunnari* feeds on *Euphausia superba*, and so this fish is directly in a higher trophic level (Carravieri et al., 2013). Gentoo penguin populations are generalists, however this individual differences in their diet confirm that some individuals are specialized in fish and others in crustaceans (Croxall et al., 1988). In addition, this may also be explained by a decrease in krill stocks. Macaroni penguins (*Eudyptes chrysolophus*) at Bird Island have more mixed diets in years of lower krill availability, particularly in 2009 (Waluda et al., 2012), which may also occur to gentoo penguin diets.

4.2 Within-individual variation of mercury levels in gentoo penguin feathers

This study is the first showing high within-individual variability in mercury levels of gentoo penguin feathers (Table II, Figure 3). There are two distinct levels of mercury in the feathers of those individuals showing variability (Figure 3), which may point to different a pattern of moult within the population. Different lengths of replacement period of all body feathers can be the main cause for this diversity, with more than one period of feather formation or a longer period of moult. During moult mercury levels decrease gradually in blood and increase in feathers. As a consequence, body feathers replaced first will display higher mercury levels than those replaced later (Bond and Diamond, 2008; Martínez et

al., 2012). Moreover, in some cases the moult can be incomplete and, as a result, this variability in feathers may represent mercury levels in successive years (Bridge, 2006; Jaeger et al., 2009; Martínez et al., 2012). Other possible reasons pointed in this subject are mercury intake during feather formation and deposition of mercury in the feathers from external routes (Martínez et al., 2012).

To understand what causes this variability, it is important to know when and how moult occurs in gentoo penguins. Gentoo penguins' breeding season at South Georgia starts in October and extends until late February, when chicks fledge. Before coming ashore to moult around mid-March, gentoo penguins forage at sea for approximately 10 days to get all the energy stores needed for this process (Davis et al., 1989). Moult can extend up to early-April, and during this period gentoo penguins fast on land and lose a significant amount of body mass (Ancel et al., 2013; Cherel, 2010; Davis et al., 1989). If their energy storage is not enough to face this process, it may reduce gentoo penguins' fitness and survival (Cherel and Freby, 1994; Cherel, 2010). Environmental variations can influence seabirds body condition and performance, mostly through fluctuations in prey availability (Baylis et al., 2012; Cherel et al., 2006). Due to the high energetic demands essential for moult, it is possible that gentoo penguins' performance on feather formation decreases in a year of low prey availability. As a result, moult may not be uniform, with a longer period of feather formation, and/or incomplete if penguins fail to replace every feather in one single moult. Given that gentoos starve when moulting, mercury intake during this period is not likely to be affecting the concentrations of this metal in feathers. Contamination by external routes during moult may not be considered either, since this metal concentrations in feathers are almost exclusively internal (Ek et al., 2004; Goede and De Bruin, 1986; Jaspers et al., 2007).

Selecting the most representative type of feather and the most trustful number of feathers in metal concentration analysis is a difficult task and depends largely on the species (Carravieri et al., 2014a; Debén et al., 2012; Head et al., 2011; Martínez et al., 2012). Body feathers are often pointed to be useful to assess mercury levels in seabirds, in opposition to flight feathers, to avoid problems with irregularity of moult (Furness et al., 1986; Thompson et al., 1993). Penguins don't have flight feathers, as these species are specialized in

diving. Therefore, in gentoo penguins only body feathers are used as they have all their body covered with the same type of feathers. Using only a few number of feathers to attain the mean mercury concentration in each individual is complicated, mainly because there is intra- and within-individual variation, but is the best way to avoid harming the birds (Cristol et al., 2012; Martínez et al., 2012).

Carravieri et al.(2014a) found low levels of within-individual variability in king penguins' (*Aptenodytes patagonicus*) feather mercury levels and suggested the use of species such as penguins, that present synchronous moult of body feathers, when monitoring mercury levels. However, it is important to notice that, as demonstrated in this study, high within-individual variation can exist in penguins. This variation in the results may lead to different trends of mercury levels within the population (Figure 3).

4.3 Temporal variation in mercury levels in gentoo penguins from Bird Island, South Georgia

The comparison between the mercury concentrations of 1998 (0.95±0.85 mg kg^{-1}; Becker et al. 2002) and the results from this study shows no variations over the previous decade (0.97±0.67 mg kg^{-1}). Although global emissions of mercury have increased from 1996 to 2006 and are predicted to double by 2050 (Streets et al., 2009), there is a time-lag between the increase of mercury levels in the atmosphere and it's deposition in the oceans. The length of this lag depends not only on global mercury cycle but also on distance from source and ocean circulation patterns (UNEP, 2013). The atmospheric concentration of mercury in the northern hemisphere is higher than in the southern hemisphere (Ebinghaus et al., 2002), due to the different levels of industrial development in these hemispheres. In the Antarctic, it is estimated that mercury levels in the atmosphere and in the oceans take 700 to 1000 years to get stability (UNEP, 2013). Hence, these 10-year results may reflect mercury levels in the atmosphere from many years before. When comparing the years of 2009 and 2010 in particular, there were biological differences in gentoo penguins breeding at Bird Island, South Georgia. In 2009, gentoo penguins breeding success was much lower than in 2010 (0.01 to 1.05, British Antarctic Survey

unpublished data) and they fed more on fish (62.2% in 2009 to 37.8% in 2011, British Antarctic Survey unpublished data). In fact, there was a decrease in krill stocks in 2009 (Waluda et al., 2012; Ward et al., 2012), which may explain the increase in the average percentage of fish in gentoo penguin diet in this year. Indeed, environmental variability may have lead to differences in prey uptake between 2009 and 2011. Although there were no differences in mercury levels between these years, it is possible that this decrease in prey availability may generate long-term effects in gentoo penguin's diet with an increase of fish consumption, which can lead to a higher accumulation of mercury in this species.

4.4 Latitudinal variation in mercury levels in feathers from gentoo penguins around Antarctica

Regarding spatial or latitudinal differences, gentoo penguins show different mercury concentrations in different breeding colonies. The lower mercury levels were found in King George Island, followed by Bird Island and Kerguelen Island, where the reported mercury levels were much higher (Table III, Figure 4).

Firstly, there may be biological differences related to diet and physiology in the different colonies. From north to south, gentoo penguin's diet tends to be less diverse and dominated by crustaceans, instead of fish (Lescroël et al., 2004). In Bird Island and King George Island, gentoo penguins feed mainly on krill and in Kerguelen Island their diet has a much larger proportion of fish (Croxall et al., 1999; Lescroël et al., 2004; Polito et al., 2011). As a result, gentoo penguins of northern colonies have higher mercury levels (Stewart et al., 1999). Brasso and Polito (2013) observed different mercury levels in two geographically separated populations of Adélie penguins (*Pygoscelis adeliae*), which they suggested to be a consequence of different trophic positions. In addition, Jouventin (1982) reported the increase of body size in gentoo penguins from south to north and recently Dinechin et al. (2012) referred that genetic differences between the Kerguelen population and those from the Atlantic are of the same range as differences between gentoo penguins and

chinstrap penguins (*Pygoscelis antarctica*). Genetic variation between populations can itself be a cause for differences in mercury levels.

Secondly, the different locations of the islands may lead to geographic barriers. These islands are situated at different latitudes, with King George Island closer to the Antarctic Peninsula at higher latitude and both Kerguelen and South Georgia Islands at lower latitudes in the sub-Antarctic, with Kerguelen Island located upon the circumpolar current. Jerez et al. (2011) found a decrease in gentoo penguin levels of lead (Pb) and other metals from north to south, which they related with the distance from anthropogenic activities. This mercury decrease along the islands can thus be explained by distance from the source. Moreover, the four times higher mercury levels in the Kerguelen Island in relation to Bird Island may be caused by its particular location, since mercury anthropogenic contamination is expected to be higher north of the Antarctic Polar Front Zone (Bargagli, 2008). Not only anthropogenic sources of mercury are important: it is necessary to take natural sources in account. Volcanic activity is the main natural source of mercury in the planet, releasing this element in similar proportions to those found in industrial areas (Bargagli et al., 1993). In the Antarctic region there are several active volcanoes, mainly in Victoria Land, Deception Island, MacDonald Island and South Sandwich Islands (Giordano et al., 2012; Mão de Ferro et al., 2014). Indeed, in Deception Island, volcanism is considered to be the main source of mercury (Mão de Ferro et al., 2014). Nevertheless, there is no evidence that volcanism in those areas is affecting South Georgia and King George Island, in relation to Kerguelen.

Table III: Mercury concentrations (mg kg^{-1}) in gentoo penguin's body feathers from different locations.

Location	Latitude	[Hg] (mg Kg^{-1})	Year	Study
King George Island	62˚S 58˚W	0.54	2004	(Santos et al., 2006).
South Georgia	54°S 38°W	0.95	1998	(Becker et al. 2002)
		0.97	2009	This study
		1.13	2011	
Kerguelen Island	49°S 70°E	5.85	2006	(Carravieri et al., 2013)
		4.96	2007	
		1.44	2006	

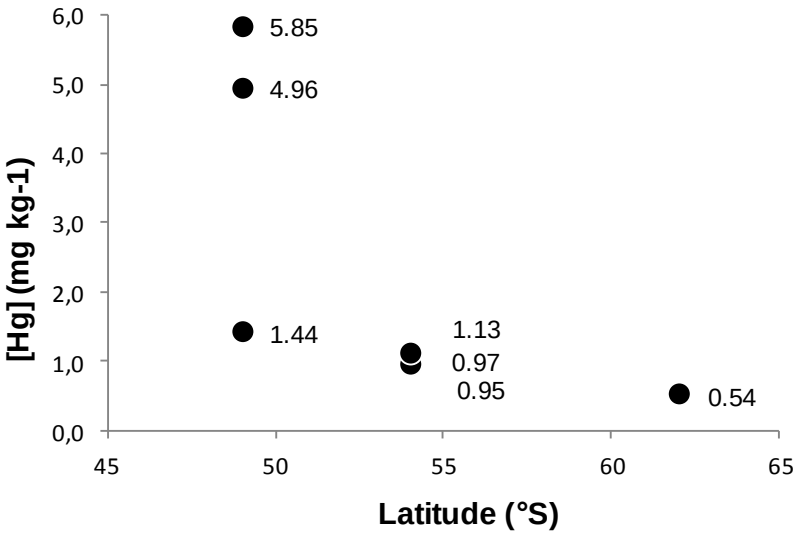

Figure 4: Mercury concentrations (mg kg^{-1}) in gentoo penguin body feathers in different locations: Kerguelen Island (49°S 70°E) (Carravieri *et al*, 2013); South Georgia (54°S 38°W) (this study; Becker *et al*, 2002); King George Island (62˚S 58˚W) (Santos *et al*, 2006).

5. CONCLUSIONS

In summary, this study highlights the significance of diet in mercury concentrations on penguins' feathers. The different rate of fish and crustaceans in their diet strongly influences the concentration of mercury in the feathers. Moreover, this study shows that using a more complete model allows the discharge of biological factors that may be correlated, namely sex and body size. The future sampling taking in account other factors may indicate more specifically other sources of mercury and in which way this pollutant is distributed in the environment. Bio-monitoring of other colonies may also contribute to show this distribution in a wider area. In addition, feather sampling should be careful, as moult patterns may influence mercury levels. Cristol et al. (2012) recommended the use of more than 4 feathers to estimate the mean mercury level per individual in bald eagles. Here, the use of at least 6 feathers per individual is suggested, in order to understand if there is more than one level of mercury in each individual.

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