Antarctic Skuas as bioindicators of local and global mercury contamination

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Abstract

Mercury (Hg) is a non-essential metal, sometimes extremely toxic, and its presence in the food-web may threaten the wildlife. In seabirds, even low levels of Hg can reduce egg production and the chances of embryos and chicks survival; high levels of this element lead to erratic behavior, loss of appetite and weight, and cellular damages in organs as kidneys can be detected. According to some authors, among 50 to 93% of the total Hg accumulated by the birds through diet can be excreted throughout the feathers during the molting process. In this way, feathers can be used as an excellent non-invasive biomonitor. The objective of this paper was to compared mercury (Hg) levels in feathers of adults and chicks of *Catharacta maccormicki* (Cma) and *C. lonnbergi* (Clo) sampled in the Antarctic Peninsula, to identify biomonitors of Hg to the region using non-invasive samples methods. We found Hg significantly higher levels in adults of Cma comparing with Clo adults ($U'=841.00$, $p<0.01$) and also with chicks of both species ($q>3.398$, $p<0.01$). We did not find significant differences comparing Clo adults and chicks of both species ($U'=16.00$, $p<0.05$), and comparing Cma breeding in different areas ($q>3.398$, $p<0.05$) or Clo ($U'= 62.00$, $p < 0.05$). The Hg levels variation may be justified mainly by differences in migration patterns. The adults of the species that migrate to more polluted areas (Cma) presented the highest levels of Hg and can be considered a promising indicator of global contamination. In another way, Clo and chicks of both species are good indicators of local Hg contamination, suffering the direct influence of contamination in the Antarctic environment.

Keywords: Biomonitoring. Pollutants. *Catharacta* skuas. Áreas de reprodução. Indicador global.

Resumo

Skuas Antárticas como bioindicadores de contaminação local e global de mercúrio
O mercúrio (Hg) é um metal não essencial, por vezes extremamente tóxico, e sua presença na cadeia alimentar pode ameaçar a vida selvagem. Nas aves marinhas, mesmo níveis baixos de Hg podem reduzir a produção de ovos e as chances de sobrevivência de embriões e filhotes. Altos níveis desse elemento levam a comportamento irregular, perda de apetite e de peso e pode causar danos celulares nos órgãos, como os rins. Segundo alguns autores, entre 50 e 93% do total de Hg acumulado pelas aves através da dieta pode ser excretado nas penas durante o processo de muda.

Dessa forma, as penas podem ser usadas como um excelente biomonitor não invasivo. O objetivo deste trabalho foi comparar os níveis de mercúrio (Hg) em penas de adultos e filhotes de *Catharacta maccormicki* (Cma) e *C. lonnbergi* (Clo) amostrados na Península Antártica, para identificar biomonitores de Hg na região usando métodos não invasivos de amostragem. Encontramos níveis significativamente mais elevados de Hg em adultos de Cma em comparação com adultos de Clo (U '= 841,00, p <0,01) e também em filhotes de ambas as espécies (q> 3,398, p <0,01). Não foram encontradas diferenças significativas de níveis de Hg na comparação entre adultos e filhotes de Clo de ambas as espécies (U '= 16,00, p <0,05) e em filhotes de Cma em diferentes áreas (q> 3,398, p <0,05) ou Clo (U' = 62,00, p <0,05). A variação dos níveis de Hg pode ser justificada principalmente por diferenças nos padrões de migração. Os adultos das espécies que migram para áreas mais poluídas (Cma) apresentaram os maiores níveis de Hg e podem ser considerados um indicador promissor de contaminação global. Por outro lado, Clo e filhotes de ambas as espécies são bons indicadores da contaminação local por Hg, sofrendo a influência direta da contaminação no ambiente antártico.

Palavras Chaves: Biomonitoramento. Poluentes. *Catharacta* skuas. Breeding sites. Global indicator.

Introduction

Mercury (Hg) is a non-essential metal, sometimes extremely toxic, and its presence in the food-web may threaten the wildlife (BLÉVIN *et al.*, 2013; GRANDJEAN *et al.*, 2010; SCHEUHAMMER *et al.*, 2007). In marine systems such as the Southern Ocean, most biologically available mercury (methylmercury) comes from current-driven transfer from coastal waters, in situ production by microbes, or from the upwelling of deep water (COSSA *et al.*, 2011). Methylmercury (MeHg) is accumulated in aquatic food chains and may become dangerous to wildlife (WHO, 1976). In seabirds even low levels of Hg can reduce the egg production and the chances of embryos and chicks survival; high levels of this element lead to erratic behavior, loss of appetite and weight, and cellular damages in organs as kidneys can be recorded (AMAP, 2002, VARIAN-RAMOS, 2014).

Considered as a "pristine" environment the primary sources of Hg in Antarctic are human activities, long term atmospheric transportation, and natural events as "summer's deglaciation" period in which Hg levels increase to rates as high as the ones observed in industrialized regions (BARBAGLI, 2008; SPROVIERI *et al.*, 2002; COSSA *et al.*, 2011). Antarctic seabirds are more exposed to Hg bioaccumulation than terrestrial animals because they are top predators feeding on organisms that concentrate inorganic Hg or MeHg phyto and zooplankton (WALSH, 1990; FURNESS; CAMPHUYSEN, 1997; BURGER; GOCHFELD, 2004; BARGAGLI, 2008).

A higher proportion of MeHg elimination in birds happens during the molt, and to a small and no significant degree during egg posture (HONDA *et al.*, 1986; *FURNESS et al.*, 1990; THOMPSON *et al.*, 1991) also, inorganic Hg could be eliminated by feces (FURNESS *et al.*, 1995). According to Burger (1993), among 50 to 93% of the total Hg accumulated by the organism through diet can be excreted throughout the feathers during molting. Another advantage of using feathers to monitor this element is that the Hg trapped on the feather suffers no influence of external deposition (VEERLE *et al.*, 2004; GOEDE; DE BRUIN, 1984) since the Hg concentration in feathers represents Hg blood levels of the organism obtained by feeding. Because of its association with keratin, the Hg inside the feathers do not change, even when these are exposed to extreme weather conditions, ultraviolet radiation, and other factors that could cause changes in the levels of Hg (APPELQUIST *et al.*, 1984). This characteristic makes feathers an excellent archive to assess the exposure of birds to Hg.

For the present study, we sampled two Antarctic seabird species, the skuas *C. maccormicki* and *C. lonnbergi*, that are top predators and have a wide distribution in the studied region (WATSON, 1975; AINLEY *et al.*, 1990; COSTA; ALVES, 2012). These species also have different migration patterns, *C. maccormicki* migrates up to the Northern Hemisphere during the austral winter, while *C. lonnbergi* features a more localized migration, remaining in the Southern Hemisphere, moving to South America and South Africa (KOPP *et al.*, 2011). The mobility of seabirds can be a disadvantage if we intend to use them as local bioindicators, but it can be an advantage if the objective is to use them as a sizeable spacial scale or global reference (BURGER; GOCHFELD, 2001).

We compared Hg levels in feathers of adults and chicks of South Polar and Brown Skua (*C. maccormicki* and *C. lonnbergi*) in three study areas of the Antarctic Peninsula, in order to assess differences in levels of contamination among species, chicks and adults and also comparing breeding areas. Since the sampled species differ in feeding and migratory habits, we tested the following hypotheses: 1. Adults of both species have different feather Hg concentrations; 2. Hg concentrations differ significantly among adults and chicks between species; 3. Adults and chicks for the same species present no significant difference in Hg levels in different breeding areas.

Material and methods

Study area and data sampling

We collected the samples in three areas of Antarctic Peninsula: Admiralty Bay (62°05'S, 58°25'W), Potter Cove (62°14'S, 58°40'W) in King George Island/Isla 25 de Mayo and at Cierva Point in Danco Coast (64°09'S, 60°58'W) (Fig. 1). The field campaign took place during the austral summer of 2007/2008 (December 2007 to January 2008), 2009/2010 (January 2010 to March 2010) and 2010/2011 (December 2010 to March 2011). The areas are divided into two regions across which the ranges of these two skuas species overlapping: the South Shetlands Islands and the Western Antarctic Peninsula (Fig. 1).

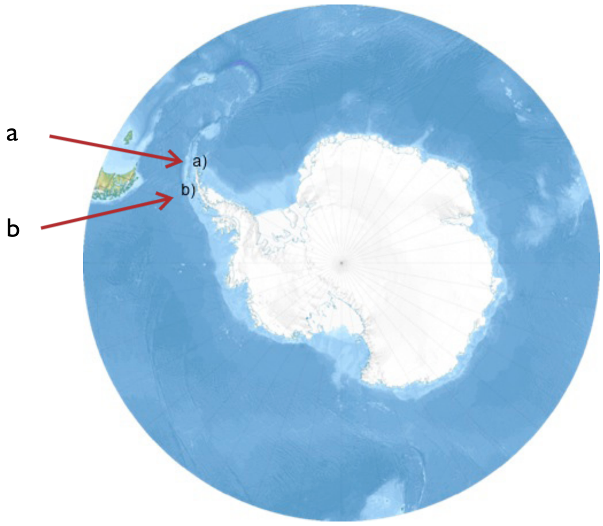

Figure 1
The map presents the Location of the South Shetlands Islands (a) and the Western Antarctic Peninsula (b).

We caught the individuals from both species – *C. maccormicki* and *C. lonnbergi* – and collected at least ten breast feathers of each bird. We stored each sample separately for each individual in labeled paper envelopes or plastic bags and kept it at room temperature until Hg analyses.

Mercury analysis

At the lab, we washed approximately 1.0 g of feathers of each bird with deionized water, EDTA (0.01%), and ultrapure water – to remove any exogenously deposited contaminations. Then we dried the samples in an oven (50°C) overnight and cut each one in small pieces (1mm). We divided the samples into three aliquots (0.33g each) and digested in 5 ml of sulphonitric solution (H2SO4: HNO3; 0,5%) for three hours in a water bath (60°C). After dissolution, we added 5 ml of potassium permanganate (KMnO4; 5%), and the samples stayed 15 min in the hot bath, then we cooled and neutralized with 1 ml of hydroxylamine hydrochloride (HO-NH3Cl+NaCl; 12%). We obtained a final volume of 12 ml by adding deionized water to the samples.

We analyzed the samples for total Hg via atomic absorption spectrophotometry by cold vapor generation (FIMS-400, Perking-Elmer) at the Laboratório de Radioisótopos Eduardo Penna Franca (Federal University of Rio de Janeiro, Brazil). Each 20 samples analyzed was preceded and followed by two blanks, a sample blank and two samples of standard reference material (IAEA 085, human hair provided by the International Atomic Energy Agency). Total Hg concentrations tare reported as parts per million (ppm) dry weight (dw). Mean percent recoveries for standard reference materials were 95.0% +- x%. The detection limit of the assay was 0.005%.

Statistical analysis

We averaged the Hg levels for adults captured twice and tested the normal distribution of data with the Kolmogorov-Smirnoff test. To compare the mean feathers' Hg levels among adults, chicks, and geographical areas, we used the non-parametric Kruskal–Wallis or Mann-Whitney tests. We also used the Dunn test to investigate differences among groups and calculated the Spearman rank correlations among variables. We presented all means followed by Standart Deviation (+-SD) and defined statistical significance at p<0.05.

Results

We analyzed Hg concentration for 122 samples of feathers for two species, including 64 adults and 34 chicks for *C. maccormicki* and 16 adults and eight chicks for *C. lonnbergi* (Table 1). For adults, the Hg average concentrations ranged from 0.65 to 9.44 mg/kg for *C. maccormicki* and between 0.46 to 2.64 mg/kg for *C. lonnbergi*. We found the highest value of Hg in an adult of *C. maccormicki*, captured in Potter Cove (9.44 mg/kg). The Hg levels for chicks were lower than for adults ranged from 0.26 to 1.56 mg/kg for *C. maccormicki* and 0.72 to 2.72 mg/kg for *C. lonnbergi*.

Table 1. Concentration of mercury (mg/kg) in breast feathers of Antarctic skuas *Catharacta maccormicki* and *C. lonnbergi* in three breeding areas in the Antarctic Peninsula. Values represent the mean ± standard deviation and sample size (n). Presented in the format: mean +- standard deviation (sample number).

Species / Age / Region	*Catharacta maccormicki*		*Catharacta lonnbergi*	
	Adults	**Chicks**	**Adults**	**Chicks**
Admiralty Bay	3.90 ± 1.20 (n=24)	0.62 ± 0.28 (n=29)	1.84 ± 0.64 (n=5)	-
Potter Cove	3.82 ± 1.99 (n=18)	1.59 ± 1.31 (n=4)	1.91 ± 0.90 (n=10)	0.88 ± 0.79 (n=8)
Cierva Point	3.64 ± 1.91 (n=24)	0.62 (n=1)	1.92 (n=1)	-

We found significantly higher Hg levels in adults of *C. maccormicki* compared with adults of *C. lonnbergi* (U' = 841.00, p<0.01) and with chicks of both species (Tukey-Kramer, q>3.398, p<0.01). We do not find significant differences comparing adults of *C. lonnbergi* with chicks of both species (U' =16.00, p < 0.05). When comparing breeding areas we had no significant differences from samples of adults of *C. maccormicki* (Tukey-Kramer q>3.398, p<0.05) or *C. lonnbergi* (U' = 62.00, p < 0.05), but we recorded statistical differences in Hg levels from chicks of *C. maccormicki* caught in different areas (U' =106.50, p<0.01). We recorded the highest Hg values for birds caught in the Potter Cove area (Table 1).

Discussion

We found no significant differences in Hg levels obtained to adults samples of the same species from different breeding sites. When we compared species, *C. maccormicki* showed higher levels of Hg than *C. lonnbergi*. This data can indicate that breeding areas in Antarctica do not represent the most significant source of Hg for adults, and other factors, e.g., diet and migration, may be associated with the differences we found. The skuas are migrant seabirds, and therefore the results from adults include not only local sources – as feathers represent the cumulative contamination during the reproductive and migratory periods the molt happens once a year for these species; (WATSON, 1975). The detailed process of molt in these birds is unknown, but it is known that the complete molt starts in late February - early March, after the breeding period, and a partial molt in head occurs at the beginning of the breeding period (WATSON, 1975). We can conclude that Hg levels found in adults' feathers of the studied skuas represent the total exposure of birds to Hg during the year, expressing Hg exposure during migratory and breeding periods.

Although the species have different diets during the breeding period, *C. maccormicki* is more generalist, feeding mainly on fish, and also in eggs and chicks of penguins and other birds, krill and carcasses of birds and mammals. In the other hand, *C. lonnbergi* feeding mainly in eggs and chicks of penguins (SANTOS *et al.*, 2012; REIS *et al.* in prep.), their diet during the austral winter (migration period) is little known and probably can be a crucial information to understand the results of the present study to *C. maccormicki*. The Hg level varied significantly between adults of two species but did not vary among offspring. The diet during the breeding period could be a variable responsible for differences in Hg levels. However, as we did not find differences among chicks receiving different diets (as mentioned species feed their chicks differently), our results did not support this. A recent paper on Hg concentration in muscles of fish Pleurogramma antarcticum, a significant prey resource in the Antarctic marine food web, suggests that predators foraging on this prey are not necessarily at a higher risk of exposure (BRASSO *et al.,* 2014). The variation presented by Brasso *et al.* (2014) for this fish ranged from 0.016 to 0.062 ppm of Hg and can present a significant variation inter-individual resulting from the broad foraging niche of adult fish. Many species, as *C. maccormicki*, rely almost exclusively on this low trophic level prey during austral summer, and this result could explain the lower Hg levels we found on offsprings of *C. maccormicki*, as the chicks are fed almost exclusively on fish.

The two species have different migration characteristics during the winter - *C. maccormicki* migrates to Northern Hemisphere, while *C. lonnbergi* remains in the Southern Hemisphere, moving to South America and South Africa (KOPP *et al.*, 2011). The migration area of *C. lonnbergi* presents lower industrial activity and, therefore, lower levels of Hg than those recorded for the Northern Hemisphere. The mobility of *C. maccormicki* can be a disadvantage when used as bioindicators on a local scale but can be a decisive factor when used on a global scale (BURGER; GOCHFELD, 2001), and they can also be useful for comparisons with no-migratory animals.

Offspring of both species presented significantly lower Hg levels than adults of *C. maccormicki*, and no differences between chicks of both species and *C. lonnbergi*, which leads us to consider that contamination recorded in chicks is an indicator of local contamination as the source of the food offered to offspring is local. Another critical factor that we need to mention is the influence of contamination transferred through the egg. Intake MeHg is fast transferred to avian eggs on a dose-dependent basis, making reproduction one of the most sensitive endpoints of Hg toxicity in birds (WOLFE *et al.*, 1998). As previously mentioned, the diet of these birds in Antarctica has less Hg available than their diet during the migration period, especially to *C. maccormicki*. Besides, many seabird species appear to be able to biotransform organic Hg into less toxic, inorganic forms that can be are stored in the body tissues (THOMPSON; FURNESS, 1989). The half-times calculated to C. skua in experimental design was all over 30 days (Bearhop *et al.* 2000) what means that until the laying period the MeHg available in the blood would have been biotransformed and stored in internal tissues – considering that the female arrives in breeding areas in early October. The laying period starts in late November. For this reason, we did not find significant differences in offspring's Hg levels for both species even when we expected to find higher levels in chicks of *C. maccormicki* as recorded for adults of this species.

We found significant differences comparing offsprings of *C. maccormicki* caught in Admiralty Bay and Potter Cove, both places in King George Island. This information indicates that we can find different levels of contamination in different breeding locations. As we did not find significant differences comparing adults in different sites, the chicks seem to be the best way to investigate local changes in the availability of Hg. We agree that the result should be viewed with caution due to the low number of chicks sampled at Potter Cove (n=4), but we strongly encourage that a more profound investigation should proceed continuously. Even small differences in concentration levels of Hg may represent a deleterious impact in individuals, especially to young. Whereas migration of *C. lonnbergi* occurs in more restricted areas with lower levels of contamination, we propose that chicks of both species and adults of *C. lonnbergi* can be used as useful indicators of local Hg contamination to monitor annual changes in the availability of this element in the Antarctic environment.

Comparing our results with those presented by Stewart *et al.* (1997) to *C. skua*, *Stercorarius parasiticus*, we found that Hg concentration is significantly higher for C. skua than for *C. maccormicki*, *S. parasiticus* and *C. lonnbergi* (ANOVA, $p<0.0001$). This information suggested that *C. skua* is more exposed to contamination than the other species analyzed, which could be explained by differences in dietary habits. The diet of C. skua is more varied than for *S. parasiticus* and includes eels, other poultry, seafood, fish, and fishery discard, while S. parasiticus feeds almost exclusively on eels (STEWART *et al.*, 1997).

The variation in Hg levels between the two sampled skuas may be justified mainly by differences in migration patterns. *Catharacta maccormicki* presented the highest levels of Hg, and it is the species that migrate to more polluted areas. Our results indicate that adults of *C. maccormicki* can be an indicator of global contamination, whereas adults of *C. lonnbergi* and chicks of both species are good indicators of local Hg contamination, suffering the direct influence of the Antarctic environment. Other factors that may be responsible for interspecific variation in mercury levels in feathers, such as body size, age, molt strategy, diet, migration patterns, physiology, and contamination in breeding or migration areas, should be investigated in future studies.

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