



Baseline

Mercury levels in feathers of Magellanic penguins

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ABSTRACT

Feathers are useful to determine mercury (Hg) contamination. We evaluated the mercury concentration in feathers of Magellanic penguins (*Spheniscus magellanicus*) age 1.5 years to 25 years at Punta Tombo, Argentina before and during their molt. Mercury ranged between <1.4 and 367 ng/g dry weight, with three extreme high values (8996 ng/g, 3011 ng/g and 1340 ng/g) all in young adults. The median concentration was lowest for juveniles and significantly higher for adults but with high variation among older adults. Males and females had similar mercury loads. Compared with other penguin species, concentrations in Magellanic penguins were low. Mercury levels for Magellanic penguins in the Southwest Atlantic for older adults averaged 206 ± 98 ng/g, and serve as a baseline for biomonitoring and/or ecotoxicological studies.

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The ocean plays an important role in the global cycle of mercury (Hg) (Fitzgerald, 1986). The presence of methylmercury in fish, birds and marine mammals is the culmination of a complex process through the food chain and can reach humans through the consumption of contaminated organisms. This process usually begins with the methylation of inorganic Hg in sediments. Methylmercury is absorbed rapidly and eliminated very slowly by the biota (D'Itri, 1990). Half-life in the body is about 2 years, generating selective enrichment compared with inorganic Hg, when moving from a lower trophic level to a higher one (UNEP, 2005 and EPA (Environmental Protection Agency) and U.S., 1997).

Studies of accumulation of Hg in birds have increased since the '60s and focus on both the toxicokinetics of the metal and the use of birds as indicators of pollution (Monteiro and Furness, 2001). As top predators, seabirds integrate exposure to contaminants over large geographic areas (Boersma, 1986; Arcos et al., 2002). To interpret what accumulated Hg levels mean, requires understanding life history, migration routes, prey, foraging habitat, and the concentration in other biomarkers or abiotic substrates (Burger and Gochfeld, 2002).

The plumage of several species of adult birds contains up to 70% of the total load of Hg in the body (Becker et al., 2002; Ochoa-Acuña et al., 2002) and almost 100% of Hg accumulated in the methylated form (Becker et al., 2002). Feathers are popular in monitoring mercury (Monteiro and Furness, 2001), because birds do

not need to be killed and sampling is minimally invasive and easy (Furness et al., 1986). Hg excretion through the quill is a detoxification mechanism for birds during the premolt (Becker et al., 2002). Once the boom ends its growth, the blood transport channel atrophies leaving a permanent record until the next molt (Burger and Gochfeld, 2002). Faced with a high environmental bioavailability of mercury, sulfur bridges of keratin may saturate, allowing free movement of methylmercury in blood to the reproductive and nervous system. Laboratory experiments show that Hg causes a wide range of effects on reproduction, including low egg weight, thinner shell, malformations of the embryo, reduced hatching, decreased growth, altered behavior and decreased chick survival (Heinz, 1979), as well as twitching, nerve damage, demyelization, and sterility (Burger and Gochfeld, 2002).

Magellanic penguins (*Spheniscus magellanicus*) are one of the most widely distributed species on the Atlantic coast of Patagonia, Argentina, occurring from the north of the Peninsula Valdés (42°04'S, 63°21'W) to the island of Tierra del Fuego (54°54'S, 67°23'W) and Falkland Islands –Malvinas– (52°S, 59°W), with Punta Tombo (Chubut) having the largest breeding colony (Boersma, 2008).

This species reproduces seasonally, attending their nests at Punta Tombo from August or early September until mid-April. Birds molt between late January and early April before migrating northward with some going as far as Brazil (Boersma et al., 1990). Their diet includes small fish such as anchovy (*Engraulis anchovy*), hake (*Merluccius hubbsi*), mackerel (*Austroatherina* sp.) and squid (*Illex* sp.) (Plan de Manejo del Area Natural Protegida Punta Tombo, 2006; Stokes and Boersma, 1998).

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Punta Tombo, one of the most valuable tourist attractions of Argentina, is visited by over 100,000 people annually (Boersma, 2008). The main environmental problems along the Patagonian coastline are associated with cities (urban and industrial effluents and solid waste disposal) and the extraction and transportation of crude oil. Most of the literature related to levels of heavy metals on these shores, refers to concentrations of Zn, Cu, Pb and Cd in abiotic compartments and at low trophic levels of the food chain (Giarratano et al., 2002; Gil et al., 1988, 1989, 1999, 2006; Harvey and Gil, 1988; Perez et al., 2005; Vázquez et al., 2007), but information on birds and mammals is scarce (Gil et al., 2006). Little is known about concentrations of Hg. It is generally assumed to be low because industrial activities releasing this metal in Patagonia are rare. However, Hg comes from natural sources such as volcanism, weathering of rocks and upwelling. Furthermore, when released in the tropics or temperate zones it is transported through the atmosphere from industrialized to remote areas, such as Polar Regions, where it accumulates in the ecosystem and food chains (Stewart et al., 1999). The only measurable concentrations of Hg for some species of birds and mammals in Patagonia are from the liver (Gil et al., 2006). In Argentina, Mercury Hg exposure of Olog's Gull from estuary of Bahía Blanca been studied by La Sala et al. (2011), but feathers of seabirds from Patagonia have never been analyzed.

We evaluated the concentration of Hg in feathers of the known-age Magellanic penguin (*Spheniscus magellanicus*) from the colony at Punta Tombo, Chubut (Fig. 1), where the age and sex of many individuals are known.

We collected feathers in 2007, between the beginning and middle of the molt. The molt lasts for approximately 3 weeks, happens once a year and while molting penguins stay on land fasting.



Fig. 1. Location of Punta Tombo (Chubut, Patagonia Argentina).

Penguin feathers were taken from 79 banded penguins in colony at Punta Tombo, Chubut, where we knew the penguin age. Juveniles were approximately 1.5 years of age and the oldest adult was 25 years of age. Approximately 5 g of feathers of each individual was plucked when the penguin was molting or feathers were cut from the chest and the back and placed in aluminum foil, labeled, and stored at -20°C . Pre-cleaning of the feathers followed Bargagli et al. (1998), using a stainless steel clamp to remove any loose foreign material collected with the feathers. We did not chemically clean the feathers. For the extraction and measurement of Hg, we used the technique described by Furness et al. (1986). We homogenized the feathers once we removed superficial sediment and biological material and weighed them before digesting them in sulfuric acid (4 ml) and nitric acid (1 ml) at 50°C for an hour. Digestion was completed by adding of 6% potassium permanganate (5 ml) also at 50°C for two hours more. After addition of hydrogen peroxide to dissolve any precipitate, we added double distilled water so each sample was 25 ml. After feathers were completely digested, total Hg concentration was determined with a cold vapor atomic absorption spectrophotometer, AAS (Hitachi Z-6100 Polarized Zeeman). Results are expressed in ng/g dry weight with a detection limit of 1.4 ng/g. The precision, expressed as the coefficient of variation of the concentrations measured on 5 replicates of a single sample, was 10.1%. From the analysis of reference material (DORM-3 Fish Protein Certified Reference Material for Trace Metals, National Research Council Canada), we recovered 92.8% of the Hg present.

We used the Shapiro–Wilk's test ($p < 0.0001$) to determine normality of variables and the Kruskal–Wallis nonparametric test ($p < 0.05$), to check for significant differences.

Mercury loads ranged between undetectable levels (<1.4 ng/g, in one juvenile) and 367 ng/g dry weight (mean 109 ± 100 ng/g, median: 73.4 ng/g). Three extreme high concentrations (8996 ng/g, 3011 ng/g and 1340 ng/g) were measured in young adults, which were excluded for statistical analysis.

Mercury levels were different between age classes. Juveniles (J) at the molt were about 1.5 years of age and had significantly lower Hg levels than those of young adults (YA) from 2 to 4 years old. Juveniles had also lower Hg levels than older breeding adults (OA) from 14 to more than 25 years old. Hg levels in young adults and the older adults were also different, (Table 1 and Fig. 2).

Males and females had similar median Hg levels in all age classes, although in adult males the range was significantly greater than in adult females (Table 2).

Concentrations of Hg in Magellanic penguins reported from internal tissues of liver ($N = 13$) from penguins found dead north of the Golfo San Jorge ranged between 0.5 and 2.2 mg/g wet weight (age not registered) (Gil et al., 2006). These values are of the same order of magnitude as those found in juveniles (about 0.5 and 3.5 mg/g wet weight, with extreme values of 30 mg/g) from the Brazilian coast (Vega Ruiz, 2008). Young penguins may go farther north than older penguins during the migration (García Borboroglu et al., 2006). The mercury levels we found are below the levels in feathers associated with negative effects on seabirds (5000 to 65,000 ng/g dry weight) (Bustamante et al., 2005; García Borboroglu et al., 2010; C.A.A, 2005; Burger and Gochfeld, 2002; Eisler, 1987). To our knowledge there are no previous studies on Hg levels in feathers of Magellanic penguins, so it was not possible to make comparisons within the species. Other species of penguins, have Hg concentrations of the same order as those we found (Table 3). However, both interspecific and intraspecific comparisons are complex given the variability in seabird life histories such as diet, timing of life events, and location of breeding and foraging grounds. Thus we do not expect similar Hg levels within and among species. One of the main changes in the concentrations of Hg in seabirds is related to their diet; they are considered top

Table 1

Average, standard deviation, median, min–max found and statistician (H) of mercury levels (ng/g, dry weight) for different age classes. Comparisons in pairs to post (J-YA $p = 0.0057$; J-OA $p < 0.0001$; YA-OA $p = 0.0154$); juveniles (J), young adults (YA), older adults (OA).

| Age classes | N | Average (ng/g) | SD (ng/g) | Median (ng/g) | Min. (ng/g) | Max. (ng/g) | H | p |
|-------------|----|----------------|-----------|---------------|-------------|-------------|------|---------|
| J | 37 | 47 | 33.3 | 52 | 1.4 | 135.1 | 32.5 | <0.0001 |
| YA | 18 | 123 | 102.7 | 77.2 | 19.9 | 319.7 | | |
| OA | 21 | 206 | 98 | 194 | 23.8 | 391.1 | | |

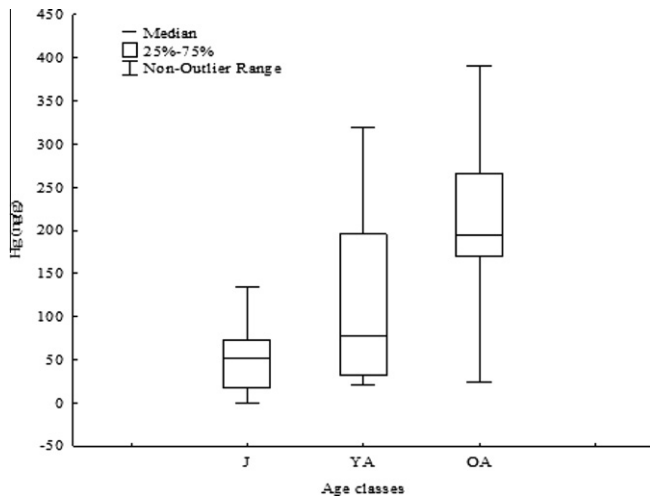


Fig. 2. Mercury concentrations (ng/g, dry weight) for different age classes (Kruskal–Wallis test, $p < 0.0001$). Extremes values are not shown in the figure.

predators in the food chain hence biomagnification of many pollutants such as Hg is expected (Vega Ruiz, 2008). Magellanic penguins feed on pelagic prey (Williams, 1995). In general, pelagic seabirds can have higher levels of mercury than shorebirds, if they feed on mesopelagic preys, because of the greater bioavailability of organic Hg (Monteiro et al., 1998). This pattern is associated with the process of methylation by anaerobic bacteria that occurs in deep water with low oxygen (Monteiro et al., 1998) and with the photodegradation of methylmercury to inorganic Hg in surface

waters (Sellers et al., 1996). The Magellanic penguin diet is opportunistic with latitudinal variation, corresponding to the distribution of prey along the continental shelf; in Argentina's Patagonian coast it is mainly piscivorous (sardine, anchovy, hake, mackerel and some cephalopods and crustaceans less frequently), (Frere et al., 1996). On the Brazilian coast their diet is mainly cephalopods (Pinto et al., 2006; Vega Ruiz, 2008). Fish can accumulate methyl mercury, especially in the edible tissue (muscle) (Perez et al., 1986) and, in general, birds eating fish may have higher concentrations than birds that feed at lower trophic levels (Vega Ruiz, 2008). In the province of Chubut, the penguins have a preference for anchovy (*Engraulis anchovy*) (Boersma, unpublished data, Williams, 1995). Total Hg obtained by Perez et al. (1986) in anchovy and hake in the Argentine Sea, yielded detectable levels (0.06 ± 0.04 mg/g wet weight and 0.11 ± 0.06 mg/g wet weight respectively), but below the permissible limit for human consumption (0.5 mg/g wet weight; Bertellotti and Yorio, 1999; C.A.A, 2005; FAO, 1973). Cephalopods live in environments with low oxygen, where the Hg methylating process occurs more easily. It enables them to easily incorporate the Hg which is transferred to their predators (Bustamante et al., 2005) including Magellanic penguins. High concentrations of total Hg (0.04 to 3.5 mg/g wet weight) are found in some species of cephalopods in the eastern Atlantic Ocean (Bustamante et al., 2005).

Magellanic penguins are vulnerable during their extensive migration. They move through areas influenced by human activities and many die of starvation when they can get caught in fishing nets or covered in petroleum (Boersma, 1987a,b; Boersma et al., 1990; Gandini et al., 1994; Garcia Borboroglu et al., 2010). The oil industry and secondary releases of mercury and other heavy

Table 2

Average, standard deviation, median, min–max found and statistician (H) of mercury levels (ng/g, dry weight) for both sexes in different age classes.

| Age classes | Sex | N | Average (ng/g) | SD (ng/g) | Median (ng/g) | Min. (ng/g) | Max. (ng/g) | H | p |
|-------------|-----|----|----------------|-----------|---------------|-------------|-------------|-----|-----|
| J | F | 16 | 52 | 34.8 | 53 | 3.9 | 135.1 | 0.4 | ns* |
| | M | 21 | 43 | 32.5 | 46 | 1.4 | 91.9 | | |
| YA | F | 7 | 125.3 | 116.8 | 76.9 | 19.9 | 319.7 | 0.1 | ns* |
| | M | 11 | 121.6 | 98.6 | 78 | 24.5 | 317.2 | | |
| OA | F | 9 | 182 | 30.6 | 191 | 105.4 | 205.2 | 0.6 | ns* |
| | M | 12 | 224 | 125.7 | 234 | 23.8 | 391.1 | | |

* Not significant.

Table 3

Review of reported of Hg concentrations in feathers in different species of penguins.

| Species of penguins | Age classes | Site | Hg (ng/g) \pm SD (N) | References |
|---|-----------------------|-----------------------|--|-------------------------|
| Adelie penguin (<i>Pygoscelis adeliae</i>) | Adults | Syowa station | 170 \pm 40 (10) | Bargagli et al. (1998) |
| Adelie penguin (<i>Pygoscelis adeliae</i>) | | Terra Nova Bay | 820 \pm 130 (3) | |
| Emperor penguin (<i>Aptenodytes forsteri</i>) | | Terra Nova Bay | 980 \pm 210 (3) | |
| Blue Penguin (<i>Eudyptula minor</i>) | Sub-Antarctic Islands | 3400 \pm 3700 (5) | | |
| Gentoo Penguin (<i>Pygoscelis papua</i>) Macaroni penguin (<i>Eudyptes chrysolophus</i>) | Age unknown | South Georgia Is | 948 \pm 848 (14) 3412 \pm 732 (20) | |
| King penguin (<i>Aptenodytes patagonicus</i>) | Age unknown | Sub-Antarctic Islands | 1980 \pm 728 (31) | Scheifler et al. (2005) |
| Adelie penguin (<i>Pygoscelis adeliae</i>) | Adults | Terra Nova Bay | Approximately 1000 (4) | Ancora et al. (2002) |
| Magellanic penguin (<i>Spheniscus Magellanicus</i>) | Older adults | Punta Tombo, Chubut | 206 \pm 98 (21) | This study |

metals in the process of cement production in the Patagonian region, could pose a risk to this species during the breeding season. However, on the shores of Punta Tombo, there are no specific anthropogenic Hg sources.

Age was an important determinant of Hg in Magellanic penguins. Levels of Hg in the internal tissues of seabirds may increase with age (Becker et al., 2002; Hindell et al., 1999), but there is no evidence that there is an increase with the years of methyl mercury concentrations in feathers (Thompson et al., 1991). One factor that could influence the concentration of Hg in each age group is their diet as variation among individuals is expected (Bertellotti and Yorio, 1999; Forero et al., 2002). Forero et al. (2002) estimated by isotope models, the contribution of anchovy in the Magellanic penguin diet varies from 46% to 76% depending on age and sex of individuals and differs among years (Boersma per. obs). Differences in diet may explain the variation in Hg concentrations among similar aged penguins. Different concentrations of Hg between the sexes are thought a result of physiological differences. Female egg laying, for example, may allow excretion of Hg, so that males would have more Hg than females (Becker et al., 2002; Monteiro and Furness, 2001). Hg levels found at the Magellanic penguin feathers at Punta Tombo, were “low” compared to values in other penguins likely reflecting the lack of mercury from human sources.

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