Science of the Total Environment 650 (2019) 1734–1739

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Humboldt penguins' feathers as bioindicators of metal exposure

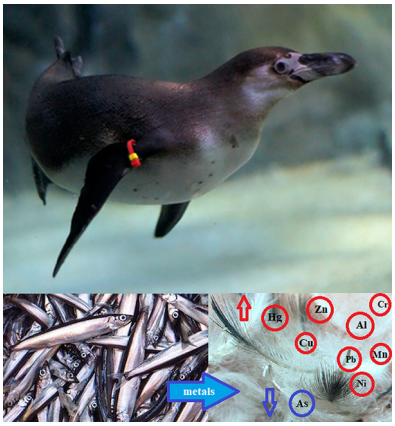
Stefania Squadrone [a,*], Paola Brizio [a], Livio Favaro [b], Gilda Todino [b], Daniela Florio [c], Cristiano Da Rugna [d], Maria Cesarina Abete [a]

[a] Istituto Zooprofilattico Sperimentale del Piemonte, Liguria, e Valle d'Aosta, via Bologna 148, 10154 Turin, Italy
[b] Department of Life Sciences and Systems Biology, University of Torino, via Accademia Albertina 13, 10123 Turin, Italy
[c] Department of Veterinary Medical Sciences, Alma Mater Studiorum Università di Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia, Bologna, Italy
[d] Acquario di Cattolica, Costa Edutainment SpA, Cattolica, Rimini, Italy

HIGHLIGHTS

- The levels of 18 metals were investigated in *Spheniscus humboldti* feathers and food.
- Al, Co, Cr, Hg, Mn, Ni, Pb, Se, Sn and Zn were higher in feathers than in food.
- Mercury concentration was in the range of moderate polluted environment.
- Penguins living indoor have lower feathers metals concentrations than penguin hosted outdoor.
- Metals levels were related to diet and environment.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 10 August 2018
Received in revised form 25 September 2018
Accepted 25 September 2018
Available online 26 September 2018

Editor: Damia Barcelo

Keywords:
Seabirds
Trace elements
Feathers
Bioaccumulation

ABSTRACT

Avian feathers have the potential to accumulate trace elements originating from contaminated food and polluted environments. In fact, in feathers, metals bind to keratin, a sulphur-containing protein for which several metals have a strong affinity. Here, the concentrations of 18 essential and non-essential elements were investigated in a Humboldt penguin (*Spheniscus humboldti*) colony housed at the Acquario di Cattolica (Italy). This species is listed as vulnerable in the *Red List* of the International Union for Conservation of Nature. According to the literature, there is usually a link between metal levels in the diet of birds and levels detected in their feathers. Thus, metals were also determined in the penguins' food (capelin, *Mallotus villosus*). We hypothesize that the controlled conditions in which birds are kept in captivity, and the homogeneous diet that they follow could allow a better understanding of metal bioaccumulation (such as mercury) or bio-dilution (such as arsenic) in the marine food chain, indicated by penguins' feathers.
Moreover, comparisons with our previous investigations performed on an ex-situ African penguin (*Spheniscus demersus*) colony suggest that penguins living indoors have lower body burden of metals than those living outdoors. Indeed, environmental contaminants usually found in areas subjected to anthropogenic impact, where zoos and aquaria are often located, are not accumulated to levels of concern.

* Corresponding author.
E-mail address: stefania.squadrone@izsto.it (S. Squadrone).

https://doi.org/10.1016/j.scitotenv.2018.09.326
0048-9697/

1. Introduction

The Humboldt Penguin (*Spheniscus humboldti*) is one of the 18 existing species of penguin and has been classified in the Red List of endangered species as having a vulnerable status by the International Union for Conservation of Nature (IUCN) (The IUCN Red List of Threatened Species, 2016).

Four species belong to this genus (*Spheniscus*), namely the Humboldt penguin, the Magellanic penguin (*Spheniscus magellanicus*), the African penguin (*Spheniscus demersus*) and the Galapagos penguin (*Spheniscus mendiculus*), which are found in South Africa and South America (Baker et al., 2006). The Humboldt penguin reproduces along the coasts and islands of Chile and Peru, from the region of Valparaiso to the Island Lobos de Tierra (Murphy, 1936). The population is composed of several thousand specimens; Chile and Peru have implemented the Washington Convention on the International Trade of wild species of endangered fauna and flora (CITES) as a national law (Paredes et al., 2003), prohibiting hunting, holding, capturing, transporting and exporting for commercial purposes (Iriarte, 1999).

Humboldt penguin colonies are also conserved and bred ex-situ in aquaria all over the world, including Italy. Penguins in zoos and aquaria are excellent model organisms to study metal bioaccumulation through food and, according to the literature, there is usually a link between metal levels in the diet of birds and levels detected in their feathers (Squadrone et al., 2018; Markowski et al., 2013; Falkowska et al., 2013a, 2013b).

For the two last decades, bird's feathers have, in fact, become one of the best choices to investigate metal pollution in natural habitats (Burger, 1993; Dmowski, 1999; Burger and Gochfeld, 2000a, 2000b, 2009; Dauwe et al., 2000; Deng et al., 2007; Burger et al., 2008; Lucia et al., 2010; Markowski et al., 2013), especially in penguins (Metcheva et al., 2006, 2011; Jerez et al., 2011; Frias et al., 2012; Carravieri et al., 2013; Lodenious and Solonen, 2013; Squadrone et al., 2016, 2018).

Concentrations of metals in bird feathers reflect the physiological state during the time of active feather growth, while metal levels in blood only reflect short-time exposure to contaminants (Burger, 1993); moreover, feather collection has the advantage of being a non-invasive method of investigation.

Thus, analysing metal levels in penguin feathers is crucial for assessing the health and welfare of captive seabirds, which could be subjected to several dietary limitations in aquaria and zoos (Squadrone et al., 2018).

In their natural habitat, penguins are predominantly piscivorous, feeding on various species of fish, small crustaceans and squid. In captivity, they usually follow a very homogeneous diet mainly composed of a single fish species, as already described by previous investigations regarding captive colonies of *S. demersus* (Falkowska et al., 2013a, 2013b; Squadrone et al., 2018).

We had the opportunity to study the metal content of feathers in Humboldt penguins at the Acquario di Cattolica (Rimini, Italy), which were exclusively fed with capelin from Norway. This is the first study aimed at investigating metal transfer of 18 trace elements from food to feathers in this species; moreover, investigations regarding metals in *S. humboldti* are very scarce. In fact, to our knowledge, only mercury levels have been investigated, by Álvarez-Varas et al. (2018) in Humboldt feathers from the Chilean and Antarctic coasts, while the concentrations of six metals (arsenic, cadmium, copper mercury, lead and zinc) have been investigated in Humboldt excreta from the northern coast of Chile (Celis et al., 2014).

This Italian Humboldt penguin colony represents a simplified marine food chain, with no interference from outdoor environments, and we aimed at testing the hypothesis that some metals, e.g. mercury, bio-magnify and are consequently present at higher levels in feathers than in fish, while others, such as arsenic, decrease as the trophic level increases in food chains. To verify this hypothesis, Humboldt penguins' feathers and food were analysed for aluminium (Al), antimony (Sb), arsenic (As), beryllium (Be), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), mercury (Hg), nickel (Ni), selenium (Se), tin (Sn), thallium (Tl), vanadium (V) and zinc (Zn).

Moreover, due to the scarcity of data regarding captive penguins, we were interested in comparing the body burden of metals in this *S. humboldti* colony, with concentrations previously found in another species of the genus *Spheniscus*, i.e. African penguins from another Italian zoological facility (Squadrone et al., 2018). The second hypothesis that we tested was to determine if the food provided to the Humboldt penguin colony had a metal content comparable to that of the food provided to the previously studied African penguin colony, then it followed that metal levels in the penguins' feathers should also have comparable concentrations.

2. Materials and methods

2.1. Sample collection

The Humboldt penguin colony (Fig. 1) was composed of 12 adult penguins (8 females and 4 males) which were housed in an indoor exhibit of the Acquario di Cattolica (Rimini, Italy), with a total area of 75 m^2, including a salt-water tank of 35 m^2 (with a maximum depth of 2 m).

Feathers were collected from moulting penguins and at the same time, penguins' food samples, capelin (*Mallotus villosus* from Norway) were also collected. All samples were pooled and stored at −20 °C for further laboratory analyses.

2.2. Analytical methods

Surface lipids and contaminants were removed from feathers as previously described (Squadrone et al., 2016, 2018). Feathers were then minced and subjected to microwave digestion utilizing an ultra-wave oven (ETHOS 1, Milestone,) with 7 mL of HNO_3 (70% v/v) and 1.5 mL of H_2O_2 (30% v/v). Mercury was quantified using a Direct Mercury Analyzer (Milestone, Shelton, CT, USA) and the other elements were measured by Inductively Coupled Plasma-Mass Spectrometry (Thermo Scientific, Bremen, Germany), following the protocols previously described (Squadrone et al., 2016, 2018). The limit of quantification (LOQ) for all elements was 0.010 mg kg^{-1}). The analytical methods were validated according to UNI CEI EN ISO/IEC 17025 (General Requirements for the Competence of Testing and Calibration Laboratories).

2.3. Statistical analysis

The unpaired two-sample *t*-test was used to compare metal levels in the feathers between the two penguin species (*Spheniscus humboldti*, in this study, and *Spheniscus demersus*, previous investigation) and in the two fish species (capelin and herring, respectively). A conservative alpha level of 0.01 was used. The Graph Pad Statistics Software Version 6.0 (GraphPad Software, Inc., USA) was used for statistical evaluations.

Results were considered statistically significant at p values of <0.01. Graph Pad Statistics Software Version 6.0 (GraphPad Software, Inc., USA) was used for statistical evaluations.

3. Results

Trace elements (in mg kg^{-1}) were found in *S. humboldti* feathers with the following decreasing mean concentrations (Table 1): Zn (50) > Fe (17) > Al (12) > Cu (11) > Hg (2.8) > Mn (2.6) > Se (0.85) > Ni (0.78) > Cr (0.54) > Pb (0.18) > As (0.14) > V (0.076) > Cd (0.060) > Sn (0.031) > Co (0.027); Be, Sb and Tl were < LOQ. In the penguins' food (capelin, Table 1) the trend was the following: Fe (11) > Zn (7.2) > As (1.8) > Cu (0.85) > Mn 0.42) > Se (0.26) > Al (0.21) > Ni (0.076) > Cd