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A comprehensive assessment of mercury exposure in penguin populations throughout the Southern Hemisphere: Using trophic calculations to identify sources of population-level variation

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ABSTRACT

The wide geographic distribution of penguins (Order Sphenisciformes) throughout the Southern Hemisphere provided a unique opportunity to use a single taxonomic group as biomonitors of mercury among geographically distinct marine ecosystems. Mercury concentrations were compared among ten species of penguins representing 26 geographically distinct breeding populations. Mercury concentrations were relatively low (⩽2.00 ppm) in feathers from 18/26 populations considered. Population-level differences in trophic level explained variation in mercury concentrations among Little, King, and Gentoo penguin populations. However, Southern Rockhopper and Magellanic penguins breeding on Staten Island, Tierra del Fuego, had the highest mercury concentrations relative to their conspecifics despite foraging at a lower trophic level. The concurrent use of stable isotope and mercury data allowed us to document penguin populations at the greatest risk of exposure to harmful concentrations of mercury as a result of foraging at a high trophic level or in geographic 'hot spots' of mercury availability.

1. Introduction

Mercury is widely distributed throughout the world's oceans; however, the rate of deposition and concentrations of mercury in the water column varies among ocean basins based on vertical and lateral circulation patterns, bathymetry, composition of coastal, shelf, and deep sea sediments, and the heterogeneity of concentrations of atmospheric mercury (Mason and Fitzgerald, 1993; Sunderland and Mason, 2007; Xia et al., 2010; Cossa et al., 2011; Mason et al., 2012). Upwelling activity, local primary productivity, and rate of *in situ* microbial production of methylmercury (the highly toxic and bioavailable form of mercury) also can drive regional scale heterogeneity in the bioavailability of mercury to marine predators in open ocean and coastal ecosystems (Sunderland and Mason, 2007; Cossa et al., 2011; Point et al., 2011). While the risk of exposure to mercury for any population certainly relates to differences in local bioavailability (Evers et al., 2007; Scheuhammer et al., 2007), these same oceanographic processes can also lead to geographic differences in prey availability to geographically distinct populations altering dietary composition and/or trophic level at the population level (Aubail et al., 2011; Bradshaw et al., 2000; Brasso and Polito, 2013; Pouilly et al., 2013). As such, mercury concentrations in a marine species may vary among geographically distinct populations. With no known biological function, the toxic effects of mercury have been shown to negatively impact ecosystem function and cause declines in bird populations at local and global scales (Braune et al., 2006; Scheuhammer et al., 2007; Evers et al., 2008). Adverse effects from exposure to mercury at the individual level manifest in the form of neurological, physiological, endocrine, immunological, and reproductive impairments in wild birds (Olsen et al., 2000; Evers et al., 2003, 2008; Brasso and Cristol, 2008; Wada et al., 2009; Jackson et al., 2011).

Seabirds are frequently used as biomonitors of mercury availability in marine ecosystems as they (1) are susceptible to biomagnification because they feed at relatively high trophic levels, (2) are long-lived and therefore prone to bioaccumulation, and (3) have wide geographic distributions (Burger and Gochfeld, 2000; Bond and Diamond, 2009). As a result of the large geographic range of

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most marine seabirds, including penguins, caution needs to be exercised when attempting to use mercury concentrations from one population as a measure of species-level risk of exposure to mercury (Evers et al., 1998, 2007; Bond and Lavers, 2011; Brasso and Polito, 2013). Rather, mercury concentrations from a single population should be interpreted as local mercury exposure within a portion of the species' range. For example, Brasso and Polito (2013) found mercury concentrations in Adélie penguin (*Pygoscelis adeliae*) chicks in the Ross Sea to be five times higher than in the Antarctic Peninsula owing to population-level differences in trophic level. A similar pattern was found between sub-populations of Gentoo penguins (*Pygoscelis papua*) breeding in the Kerguelen Islands in which a fivefold difference in mercury was driven by variation in foraging habits (Carravieri et al., 2013). As a growing number of studies are beginning to address mercury exposure in penguins throughout the Southern Hemisphere (Brasso et al., 2012; Blévin et al., 2013; Brasso and Polito, 2013; Carravieri et al., 2013) we identified a need for an integrative, comparative assessment to better understand the causes of intra- and inter-specific differences in mercury exposure within this group of seabirds.

Owing to the biomagnification of mercury and the general correlation between $\delta^{15}N$ values and trophic level (Jardine et al., 2006), stable isotope analysis can be a useful tool for comparing mercury concentrations among sympatric species in marine and aquatic food webs (Chasar et al., 2009; Brasso et al., 2012; Day et al., 2012; Carravieri et al., 2013). However, $\delta^{15}N$ values cannot be directly compared among geographically distinct food webs as temporal and spatial variation in primary productivity, latitude, and ocean frontal region results in geographic differences in baseline $\delta^{15}N$ values (Post, 2002; McMahon et al., 2013). To account for this, population-specific trophic levels can be calculated by pairing consumer and ecosystem baseline $\delta^{15}N$ values within a given ecosystem. The calculation of a population-specific trophic level allows for trophic comparisons across large geographic scales and is particularly useful in large-scale ecotoxicological studies (Day et al., 2012; Brasso and Polito, 2013). Thus, trophic calculations allow for the direct comparison of mercury exposure among geographically distinct populations and, in the absence of trophic disparities, for the identification of possible 'hot spots' of mercury availability within a species range (e.g. Point et al., 2011; Brasso and Polito, 2013).

The goals of the present study were threefold. First, we review, update, and in some cases, provide the first data on mercury concentrations for ten penguin species breeding throughout the Southern Hemisphere. To achieve this goal we combined adult feather mercury concentrations from the literature with new data from adult body feathers collected from eight species of penguins (from 10 populations) breeding in distinct marine ecosystems across the Southern Hemisphere. Second, for populations in which stable isotope values ($\delta^{15}N$) were available, population-specific trophic levels were calculated to determine the source (dietary or environmental) of variation in mercury using the hypothesis testing framework described by Brasso and Polito (2013). Briefly, when calculated trophic levels are similar among populations, but mercury concentrations differ (or vice versa) it supports the hypothesis that the difference in mercury between populations is the result of disparities in the bioavailability of mercury between ecosystems. Alternately, the use of different foraging habitats between geographically distinct populations could also lead to disparities in mercury exposure in the absence of trophic level variability. On the other hand, if trophic level differences are mirrored by differences in mercury concentrations among populations it supports the hypothesis that trophic disparities account for observed differences in mercury exposure between populations. Population-specific trophic levels were calculated using mean feather $\delta^{15}N$ values either determined here or derived from the literature to generate the largest assessment of mercury exposure in penguins to date. Finally, we provide the first documentation of penguin populations at risk of exposure to elevated concentrations of mercury as a result of foraging in geographic mercury 'hotspots' or at elevated trophic positions.

2. Methods

2.1. Sample collection

Collection of adult body feathers from penguins occurred in the following marine ecosystems in the Southern Hemisphere: Southern Ocean (Antarctic Peninsula and South Georgia), southern Patagonian shelf large marine ecosystem (Le Maire Strait, Staten Island, and Beagle Channel), Benguela upwelling ecosystem (coastal South Africa), and the Bass Strait (southeastern Australia; Fig. 1). Body feathers were collected from ~20 adult individuals/year from eight species of penguins at 10 geographically distinct locations during the 2008–2013 breeding seasons (Supplementary Table 1). Samples from Gentoo (*P. papua*) and Magellanic (*Spheniscus magellanicus*) penguins were each collected from two geographically distinct populations; for clarity, these species are presented with an abbreviation specifying the location of each population: AP (Antarctic Peninsula) and SG (South Georgia) for Gentoo penguins, IM (Isla Martillo) and SI (Staten Island) for Magellanic penguins. Feathers were collected from random locations on the bodies (dorsal and ventral; below the neck and above the lower extremities) of adult birds captured during nest surveys, banding, or during rescue/rehabilitation efforts under appropriate animal handling and collection permits. Sex in *Pygoscelis* penguins in the Antarctic Peninsula, Magellanic penguins from Staten Island and Isla Martillo, and Southern Rockhopper Penguins (*Eudyptes chrysocome*) from Staten Island was determined using comparative morphometrics (bill depth and length; Gandini et al., 1992; Hull, 1996; Polito et al., 2012). Sex was not determined in King (*Aptenodytes patagonicus*), African (*Spheniscus demersus*), and Little (*Eudyptula minor*) penguins. Adult feathers were stored separately for each individual in sealed plastic bags and were kept at room temperature until mercury analysis.

2.2. Mercury analysis

Due to the relatively low intra-individual variation of mercury in penguin body feathers a single feather from each individual was considered sufficient for analyses of total mercury (Brasso et al., 2013). Each feather sample was rinsed in a series of six alternating vials of acetone and deionized water to remove any exogenously deposited oils or contaminants. Feathers were allowed to dry under a fume hood for ~24 h and were subsequently stored in clean zip-top bags at room temperature until mercury analysis. Whole, individual body feathers were analyzed separately for total mercury via atomic absorption spectrophotometry on a Tri-Cell Direct Mercury Analyzer (DMA-80) at the University of North Carolina Wilmington (Wilmington, NC, USA). Because nearly all mercury in feathers is present in the form of methylmercury, a measurement of total mercury concentration was used as a proxy for this highly bioavailable form (Bond and Diamond, 2009). Analysis of each set of 20 samples analyzed was preceded and followed by two method blanks, two sample blanks, and two samples each of standard reference material (DORM-3, DOLT-4; fish protein, and dogfish liver certified reference materials, respectively, provided by the National Research Council Canada). All mercury concentrations are reported as parts per million (ppm) fresh