

## Short Communication

## UNIQUE PATTERN OF MOLT LEADS TO LOW INTRAINDIVIDUAL VARIATION IN FEATHER MERCURY CONCENTRATIONS IN PENGUINS

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**Abstract:** The authors hypothesized that the catastrophic annual molt of penguins (Sphenisciformes) would lead to reduced intraindividual variation of mercury concentrations in body feathers. While mean mercury concentrations varied significantly among 8 penguin species, intraindividual variability did not differ among species and was 3 times lower than values observed in other seabirds. The findings of the present study suggest that a single body feather collected at random per individual can be adequate to estimate mercury exposure at the population level in penguins. *Environ Toxicol Chem* 2013;32:2331–2334. © 2013 SETAC

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## INTRODUCTION

As long-lived predators foraging at high trophic positions, in many cases as obligate piscivores, seabirds are at an elevated risk of exposure to dietary mercury and are therefore one of the most commonly utilized biomonitors of mercury availability in marine and aquatic ecosystems [1]. Seabird feathers are typically chosen as the monitoring unit because they accurately reflect dietary uptake and body burden of mercury at the time of molt, providing a proxy for the availability of mercury in a given ecosystem [2–4]. Collecting feathers is relatively noninvasive, causing little disturbance to the bird, and feathers are easily stored and transported after field collection [3]. Furthermore, at least 95% of the mercury in feathers is present as methylmercury, the concentration of which is extremely stable within the feather matrix across a variety of environmental conditions and through time [2,5,6]. To accurately interpret feather mercury concentrations, however, the timing and sequence of feather growth must be carefully considered.

Due to the sequential molt of flight feathers and variation in the timing of body feather molt, significant intraindividual variation in feather mercury has been documented in several species of flying seabirds and raptors, signifying that molt sequence must be considered to account for potentially high intraindividual variation [2–4,7]. However, this concern may not apply to penguins. Penguins (order Sphenisciformes) have a unique plumage and a distinctive annual molt (catastrophic molt) in which all body feathers are lost and regrown over a 2-wk to 3-wk period of fasting [8–10]. The loss of body feathers typically occurs in a course reflecting preening and abrasion from movement about the colony, with large quantities of feathers being sloughed at once [8].

The plumage of penguins is unique in that, lacking pterygiae, feathers cover the entire body—a likely thermoregulatory adaptation to prolonged foraging bouts in cold water [9]. While

body feather length can vary slightly among species in terms of body size or geographic distribution (polar vs temperate species), all body feathers within an individual are of approximately the same lancelet shape and size [9]. The average difference in size between the largest and smallest body feathers on an individual is 3 mm to 5 mm across all species [9]. Because of the notable absence of flight feathers and similarly sized, concurrently grown body feathers, mercury concentrations in individual feathers throughout the plumage of penguins may not be as variable as in other seabirds in which flight and body feathers are of considerably different sizes and are grown over extended periods (from months to years, such as in the family Diomedidae) [11].

Given the high intraindividual variation of mercury in seabird body feathers, sampling multiple body feathers from each individual is required to accurately assess body burden [4]. However, access to numerous samples per individual may not be feasible when one is sampling from tissue archives (e.g., museums, universities). Tissue collections are often shared resources, and so utilizing the minimum amount of tissue per individual is ideal to maximize the number of analyses that can be performed while maintaining the integrity of the collection. Penguin feathers could be a good model for assessing mercury concentrations using a minimum number of samples, as all of their body feathers are grown concurrently and are of a similar size. In the present study, we hypothesized that the catastrophic molt pattern of penguins would lead to relatively low intraindividual variation in body feather mercury, allowing for a more conservative use of tissues to assess an individual's body burden of mercury. If mercury concentrations in penguin body feathers prove less variable than in other seabirds, penguin body feathers may serve as a more effective tool for monitoring mercury in marine ecosystems throughout the Southern Hemisphere.

## METHODS

Five body feathers were collected from 10 individuals from 8 species of penguins across the Southern Hemisphere during the

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2008 to 2012 breeding seasons (Table 1). 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 rescue or rehabilitation efforts, under appropriate animal handling and collection permits. Body feathers were stored separately for each individual in a zip-top bag and were kept at room temperature until mercury analysis.

Body feathers were cleaned by rinsing in 6 alternating vials of acetone and deionized water to remove any exogenously deposited oils or contaminants. Feathers were left to air dry for approximately 24 h under a fume hood, sorted into clean zip-top bags, and stored at room temperature until mercury analysis. Whole, individual body feathers were analyzed directly 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 [6]. Each set of 20 samples analyzed was preceded and followed by 2 method blanks, a sample blank, and 2 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 weight (fresh wt). Mean recovery percentages for standard reference materials were  $100.3\% \pm 1.5\%$  (DORM-3) and  $98.9\% \pm 0.6\%$  (DOLT-4). The relative significant difference for both standard reference materials was  $<2.1\%$  throughout duration of the present study.

The coefficient of variation (CV) [(standard deviation  $\times$  100)/mean] was calculated for each individual as a measure of intraindividual variation of mercury in body feathers ( $n = 5$  feathers per individual). Lower CVs were equated with low intraindividual variation, and higher CVs indicated a higher degree of intraindividual variation in mercury. Univariate analyses of variance (ANOVAs) were used to compare mean feather mercury concentrations (average mercury concentration of feathers 1–5 from an individual) and CVs among species. A paired  $t$  test was used to compare feathers with the minimum and maximum mercury concentrations within an individual. To determine whether the difference percentage [(maximum – minimum/maximum)  $\times$  100] between the feather with the highest and the lowest mercury concentration (within an individual) varied across species, we used an analysis of covariance (ANCOVA) with mean feather mercury (for each individual) as a covariate. To investigate whether body size had an effect on mean feather mercury concentration or CV, a body mass index (BMI; [mass(kg)/length<sup>2</sup>(m)]) was calculated for

each species using data available in the literature [12]. Data for males and females were averaged to utilize a single value for each species in all subsequent analyses. Pearson's correlations were then used to determine whether CVs were correlated with BMI and minimum, maximum, and mean feather mercury concentrations. Log-transformed mercury concentrations were used in all statistical comparisons (exclusive of the bootstrap technique described below, because that technique does not assume an underlying distribution) to generate data with a more normal distribution (Kolmogorov–Smirnov,  $p = 0.60$ ). All means are presented  $\pm$  standard deviation (SD); statistical significance was defined at  $p < 0.05$ . Statistical comparisons were conducted using SPSS (Ver 18.0).

Finally, we used a bootstrap technique [13] to test the null hypothesis that the number of feathers sampled from the individual birds had no effect on our ability to distinguish differences in mercury concentrations among species (R Ver 2.15.1). This procedure started by averaging a sample (with replacement) of  $s = 1, 2, 3, 4,$  or  $5$  of the original feathers for each individual bird ( $i = 1, 2, \dots, 10$ ) in each species ( $j = 1, 2, \dots, 8$ ) to estimate mercury concentration at the individual level. We resampled 10 000 times to characterize the possible sample space, where each  $k = 1, 2, \dots, 10$  000 trial generated an estimate of individual mercury concentration,  $y_{ijk}$ . Then, for a given sample size  $s$  and trial number  $k$ , the average species-level estimates of mercury concentration were

$$\bar{y}_{\cdot jk} = \sum_{i=1}^{10} y_{ijk} / 10$$

We then calculated the sum of squared deviations between the species-level concentrations and the grand mean

$$SSD(s)_k = \sum_{j=1}^8 (\bar{y}_{\cdot jk} - \bar{y}_{\cdot \cdot k})^2$$

to characterize the dispersion among species when the mercury concentration from  $s$  feathers was averaged. Finally, to test our hypothesis, we constructed a test statistic by finding the ratio of 2  $SSD(s)_k$  with different feather sample sizes  $s$ . For example, the following is the of among-species variation estimated in a trial averaging 5 and 1 feathers per individual, for a total of 10 pairwise comparisons

$$\rho_k = SSD(5)_k / SSD(1)_k$$

If there was no statistically significant difference in the estimated dispersion among species, we would expect this ratio to be near 1 ( $\rho_k - 1$ ). Thus, if the 95% confidence intervals (CIs) of

Table 1. Study sites and mean body feather mercury (Hg) concentrations (ppm, fresh wt) for 8 species of penguins<sup>a</sup>

Region	Site	Latitude, longitude	Common name	Species	Mean Hg $\pm$ SD
Antarctic	King George Island	62°10'S, 58°27'W	Adélie	<i>Pygoscelis adeliae</i>	0.32 $\pm$ 0.08 A
			Chinstrap	<i>Pygoscelis antarctica</i>	0.69 $\pm$ 0.18 B
			Gentoo	<i>Pygoscelis papua</i>	0.28 $\pm$ 0.05 A
Sub-Antarctic	South Georgia	54°26'S, 36°11'W	King	<i>Aptenodytes patagonicus</i>	2.92 $\pm$ 0.76 E
South Africa	Robben Island	33°48'S, 18°22'W	African	<i>Spheniscus demersus</i>	0.90 $\pm$ 0.35 C
South America	Isla Martillo	54° 53'S, 67° 34'W	Magellanic	<i>Spheniscus magellanicus</i>	1.67 $\pm$ 0.22 D
	Staten Island	54° 50'S, 64°40'W	Rockhopper	<i>Eudyptes chrysocome</i>	5.44 $\pm$ 0.67 F
Australia	Phillip Island	38° 31'S, 145° 07'E	Little	<i>Eudyptula minor</i>	2.92 $\pm$ 2.88 E

<sup>a</sup>Mean mercury concentrations that do not share an uppercase letter were found to be significantly different based on Tukey's honest significant difference test ( $p < 0.05$ );  $n = 10$  individuals per species. SD = standard deviation.

the distribution of  $\rho_k$  values included 1, then there was insufficient evidence to reject the null hypothesis at a critical value of 0.05, which would match our expectation.

## RESULTS

Body feather mercury concentrations varied significantly among species (ANOVA:  $F_{7,72} = 108.69$ ,  $p < 0.001$ ; Table 1). Regardless of mean mercury concentration, CVs were relatively low and did not differ significantly among species (ANOVA:  $F_{7,72} = 1.82$ ,  $p = 0.10$ ; Figure 1.). Overall, CVs were not correlated with maximum ( $r = -0.11$ ,  $p = 0.34$ ) or mean feather mercury concentrations ( $r = -0.19$ ,  $p = 0.10$ ), although a weak correlation was detected between CV and minimum mercury concentration ( $r = -0.26$ ,  $p = 0.02$ ). Furthermore, no significant correlations were found between BMI and CV ( $r = -0.17$ ,  $p = 0.14$ ) or mean mercury concentration ( $r = 0.05$ ,  $p = 0.65$ ).

Despite relatively low intraindividual variation, a significant difference was detected between the minimum and maximum feather mercury concentration at the individual level (paired  $t$  test:  $t = -13.06$ ,  $df = 79$ ,  $p < 0.001$ ). Across all species the average maximum difference percentage between feathers with the highest and lowest mercury within an individual was  $21.3\% \pm 12.3\%$ ; the minimum difference percentage between any 2 feathers from an individual was  $7.82\% \pm 8.66\%$ . Overall, interspecies differences in the maximum difference percentage were not statistically significant (ANCOVA:  $F_{7,71} = 2.04$ ,  $p = 0.06$ ). Furthermore, there was no effect of mean body feather mercury on the maximum difference percentage within an individual (ANCOVA:  $F_{7,71} = 2.88$ ,  $p = 0.09$ ).

The results of the bootstrap technique showed no significant effect of the number of feathers sampled from an individual on the estimated among-species variation in mercury concentration. In all 10 pairwise comparisons, the 95% CIs of our test statistic  $\rho_k$  included 1 (lower CIs = 0.873–0.928, upper CIs = 1.078–1.145). Furthermore, despite different average concentrations of mercury among species, the estimated mean mercury value for each species changed little when 1, 2, 3, 4, or 5 feathers from each individual within a species were sampled (Figure 2). As expected, the width of the 95% CIs of the estimated species-level mercury concentrations declined as the number of feathers sampled increased.

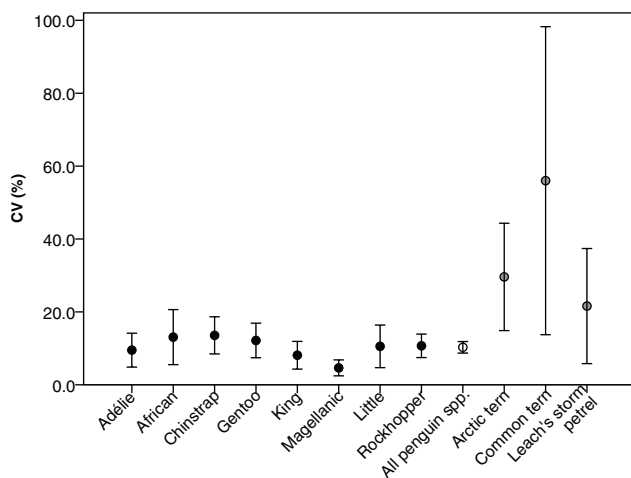


Figure 1. Mean coefficient of variation (CV) for each species of penguin (black circles;  $n = 10$  individuals per species), all penguin species combined (open circle), and reported literature values for flying seabirds (gray circles;  $n = 5$  individuals per species; [4]). Error bars indicate  $\pm$  standard deviation.

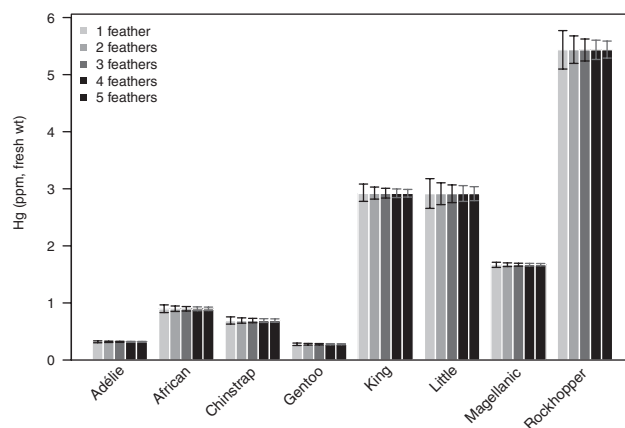


Figure 2. Estimated mercury (Hg) concentrations in 8 species of penguins generated with a bootstrap resampling procedure using 1 to 5 feathers from each of 10 individuals to generate a species-level mean. Each bar on the graph represents the mean ( $\pm$  95% confidence interval) of 10 000 resampling events.

## DISCUSSION

To our knowledge, the present study is the first to document relatively low intraindividual body feather mercury concentrations in a seabird—the likely result of the unique catastrophic molting strategy of birds in the order Sphenisciformes. Although a significant difference was detected between the minimum and maximum mercury concentrations within an individual, the maximum difference percentage in penguins was approximately 10% lower than the minimum difference percentage reported in flying seabirds [6]. The mean CV did not vary significantly among penguin species, nor was there a correlation between mean feather mercury and CV, suggesting that variation in body feather mercury is not related to body burden of mercury. While body feather mercury concentrations can vary within an individual (8–20% on average), our bootstrap procedure suggests that a representative mean mercury concentration for a given population or species can be attained using a single body feather collected at random from each individual, given a sample size of at least 10 individuals per group.

Bond and Diamond [6] investigated intraindividual variation in 3 species of seabirds—Arctic tern (*Sterna paradisaea*), Common tern (*Sterna hirundo*), and Leach's storm petrel (*Oceanodroma leucorhoa*)—and found an average CV of  $35.7\% \pm 25.5\%$  across all species, a likely result of the random or protracted nature of body feather molt in seabirds. Common terns had the highest CV, averaging  $56.0\% \pm 34.0\%$ , with 1 individual documented with a CV of 100% ( $n = 5$  feathers from each of 5 individuals) [4]. The intraindividual variation in penguins was, on average, one-third of that found in these 3 species of seabirds; the Chinstrap penguin had the highest CV, with an average of  $13.5\% \pm 7.1\%$ . Furness et al. [2] found moderate variation in body feathers in a great skua ( $18.5\text{--}28.6\%$  among belly, back, and breast; *Catharacta skua hamiltoni*) and a kittiwake ( $6.5\text{--}12.5\%$  among belly, back, and breast; *Rissa tridactyla*); however, only 1 individual was analyzed per species.

The variation in body feather mercury in seabirds has also been hypothesized to be a product of changes in a bird's metabolic rate or foraging during the often protracted period of body feather growth [4]. Any change in foraging location, diet, or prey mercury concentration during the feather growth period could be reflected in the body feathers, especially if individual

feather growth occurs over the course of days or weeks [3,4]. However, all species of penguin remain at their molt site and fast during the 2-wk to 3-wk molt period [8,10]. Thus, feather mercury concentrations can only reflect dietary uptake prior to molt and body burden as penguins utilize endogenous nutrient stores to survive this prolonged period of fasting and feather growth [10,14]. Fasting and utilizing endogenous stores of nutrients during feather growth may explain the limited variability in body feathers of penguins.

The use of body feathers for determining mercury concentrations has been suggested as an alternative to primary flight feathers, as significant variation has been documented in which mercury concentrations in primaries grown first are significantly higher than in flight feathers grown later in the sequence [2,7,15]. This pattern in flight feathers is thought to be the result of a reduction in body burden throughout the molt sequence related to the sequestration of mercury into feathers grown earlier in the molt cycle [2]. To this end, the random sequence and protracted timing of body feather molt in many seabirds causes significant variation in body feathers within an individual as well [2,4]. High intraindividual variation in body feather mercury could lead to significant over- or underestimates of body burdens of mercury in the absence of standardized feather collection protocols in which multiple feathers per individual are collected. Despite previous documentation of this phenomenon, no sampling protocols currently exist for seabirds; and due to species-specific molt patterns, this may need to be examined at the family or genus level.

Likely related to the relatively low variation in mercury at the individual level, our bootstrap procedure suggests that a single feather per individual may be used to estimate mercury exposure at the species level in the family Spheniscidae. While increasing the number of feathers sampled from an individual did decrease the 95% CIs of the estimated mean concentrations, we were able to detect the differences among the species with sufficient confidence even when using only 1 randomly sampled feather per individual. Similar bootstrap procedures may be useful for species with higher levels of intraindividual variation to establish the minimum number of samples needed to accurately establish species-level estimates of mercury. This may be of particular importance in terms of avoiding over- or underestimates of mercury exposure in a given species. Finally, as the intraindividual variation in penguins has proved to be significantly lower than variation reported in other seabirds, we suggest that penguins may serve as more accurate biomonitors of mercury in marine food webs in the Southern Hemisphere.

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