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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 ($\leqslant$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 δ^{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, δ^{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 δ^{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 δ^{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 (δ^{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 δ^{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

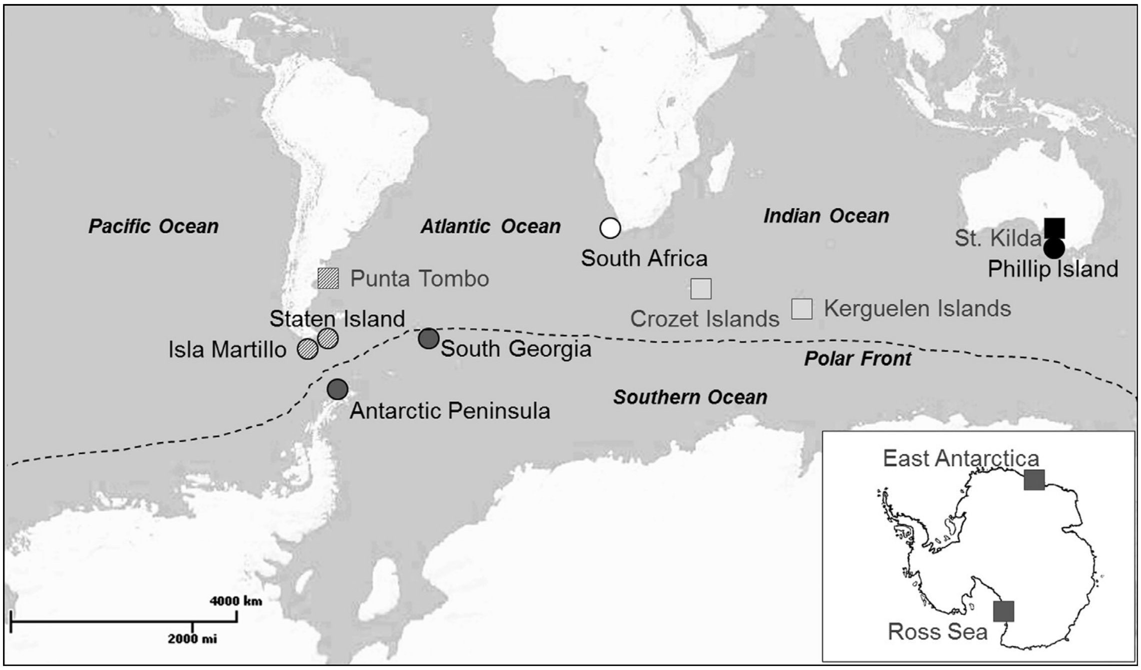

Fig. 1. Locations of penguin populations used in trophic comparisons; circles represent field-sampled locations, square symbols represent populations with literature derived data. Adult penguin feathers collected from: Isla Martillo and Staten Island, Tierra del Fuego, Argentina (southern Patagonian large marine shelf ecosystem; hashed), King George Island, South Shetland Islands, Antarctic Peninsula and Gold Harbor, South Georgia (Southern Ocean; dark grey), Robben Island, South Africa (Benguela upwelling ecosystem; white), and Phillip Island, Victoria (Bass Strait; black). Penguin populations from which mercury data were literature derived (grey text, square symbols): St. Kilda, Port Phillip Bay, Australia; Punta Tombo, Brazil; Crozet and Kerguelen Islands; and Ross Sea and East Antarctica (inset map of Antarctica).

weight (fresh wt). Mean recovery percentages for standard reference materials were 100.3% ± 1.5% (DORM-3) and 98.9% ± 0.6% (DOLT-4). The relative significant difference for both standard reference materials was <2.1% throughout the assay.

2.3. Trophic level calculations

A trophic level for each species was calculated from tissue $\delta^{15}N$ values using a modification of the model described by Hobson et al. (1994, 2002):

$$TL_{consumer} = 2 + (\delta^{15}N_{consumer} - \delta^{15}N_{primary\ consumer})/\Delta_{N\ food\ web} \quad (1)$$

This model uses a consumer's $\delta^{15}N$ value to estimate its trophic level relative to the mean $\delta^{15}N$ value of a primary consumer (assumed trophic level of 2) and the mean ^{15}N food web trophic discrimination per trophic transfer (Δ_N). However, tissues from field collected samples and literature derived data used here varied (feathers and blood) and $\delta^{15}N$ values in seabird tissues are dependent on tissue-specific discrimination factors (Bond and Jones, 2009; Polito et al., 2009). Therefore, we modified formula (1) to standardize calculations between tissues by adding an additional term to account for tissue-specific dietary isotopic discrimination factors as proposed by Hobson and Bond (2012):

$$TL_{penguin} = 3 + (\delta^{15}N_{consumer} - \Delta_{N\ avian\ tissue} - \delta^{15}N_{primary\ consumer})/\Delta_{N\ food\ web} \quad (2)$$

Using formula (2) we incorporated mean penguin isotopic values for $\delta^{15}N_{consumer}$ and food web-specific primary consumer isotopic values for $\delta^{15}N_{primary\ consumer}$. Published discrimination factors ($\Delta_{N\ avian\ tissue}$) for penguin feathers (+3.5‰, *Pygoscelis* penguins, Polito et al., 2012; +4.4‰, Southern Rockhopper penguins, Cherel et al., 2005) and blood (+2.7‰, for all species except King penguins (+2.1‰); Cherel et al., 2005) were applied to the equation dependent upon the tissue used to provide $\delta^{15}N_{consumer}$ values. For the *Pygoscelis* penguins in the Antarctic Peninsula, $\delta^{15}N$ values were determined in feathers collected from the same individuals sampled for mercury analysis from King George Island (see Polito et al., 2011 for analytical methods). Individual $\delta^{15}N$ values were used to calculate a mean TL_{bird} for each of the three pygoscelid species. Trophic levels for all other populations except King and Magellanic penguins from Isla Martillo were calculated using literature derived mean $\delta^{15}N_{consumer}$ and $\delta^{15}N_{primary\ consumer}$ values (Supplementary Table 2). Tissue $\delta^{15}N$ values were not available for Magellanic penguins at Isla Martillo or for King penguins; thus, trophic level was estimated from dietary composition. To estimate trophic level (TL_i) for these populations we used the following formula from Pauly et al. (1998), incorporating available data on dietary composition for each species:

$$TL_i = 1 + \left(\sum_{j=1}^{8} TL_j \cdot DCij \Big/ \sum_{j=1}^{8} DCij \right) \quad (3)$$

where TL_j represents the mean trophic level of prey species *j* and DC*i* is the proportion of the diet made up by prey species *j*. The dietary composition of King penguins at Fortuna Bay, South Georgia, was described from stomach contents of breeding adult birds by Olsson and North (1997). The diet of King penguins consisted of approximately 97% fish, most of which were in the family Myctophidae (TL = 3.8, *Krefftichthys anderssoni*, Stowasser et al., 2012), with the remaining 3% consisting of squid (TL = 3.7, *Galiteuthis glacialis*, Stowasser et al., 2012). A similar approach was taken in estimating the trophic level of Magellanic penguins breeding at Isla Martillo. At this location Magellanic penguins fed mainly on fuegian sprat (TL = 4.1, *Sprattus fuegensis*), representing 55% of the biomass consumed by birds (Scioscia et al., 2014). The next most important prey item was squat lobster 27 % (TL = 2.7, *Munida gregaria*) followed by Patagonian squid 14% (TL = 3.8, *Loligo gahi*) and 4% other prey (Scioscia et al., 2014). The proportion of each prey type in the diet along with the trophic level of common prey species were incorporated into formula (3), above, to estimate trophic level of King and Magellanic penguins (IM).

It is important to note the timing of tissue incorporation of diet ($\delta^{15}N$) relative to mercury exposure in the tissues used in the present study. Feather $\delta^{15}N$ values and mercury concentrations in adult penguin feathers reflect an integrative signal of endogenous tissue mercury (inter-molt period) and diet prior to fasting (Evers et al., 2008; Bond and Diamond, 2009; Bond and Jones, 2009). It should be noted that for 9/20 populations, trophic level was calculated from blood $\delta^{15}N$ values as feather $\delta^{15}N$ values were unavailable in the literature (see Supp Table 2). For these populations, the trophic level calculated may only provide a seasonal, rather than annual, estimate of diet.

2.4. Literature review

To extend our population-level comparison, we conducted a systematic review of the literature for penguin populations for which both adult feather mercury concentrations and $\delta^{15}N$ values (penguin tissue and primary consumer) have been published to allow for the calculation of trophic level for comparison with our data set (Supplementary Table 2). For consistency, all trophic level calculations using literature-derived mean $\delta^{15}N$ values were conducted using the modified equation from Hobson and Bond (2012) described in formula (2) above. Here, adult feather mercury concentrations are reported from 26 populations of penguins from five geographically distinct marine ecosystems across the Southern Hemisphere (ten populations sampled in the field, 16 populations using literature-derived data). Of these populations, we were able to calculate trophic level for the ten field-sampled populations and for ten of the literature-derived populations (Table 1).

2.5. Statistical analysis

Statistical calculations were performed using SPSS (PASW Statistics 18.0, 2009). Log transformed adult feather mercury concentrations were used in statistical comparisons to produce data with more normalized distributions, as determined by Shapiro Wilkes tests. Two-way student's *t*-tests were used to compare mean mercury concentrations among penguin populations using field collected data or literature derived means (when ±SD and sample sizes were available). Pearson's correlation was applied to examine the relationship between mean mercury concentrations and calculated trophic levels among all 20 penguin populations for which both mercury concentrations and trophic levels were available. A two-way ANOVA was used to examine the effect of sex on mercury concentrations in the following populations: all three species of *Pygoscelis* penguins in the Antarctic Peninsula, Magellanic and Southern Rockhopper penguins at Staten Island, and Magellanic penguins at Isla Martillo. This ANOVA included

Table 1
Mean adult feather mercury concentrations (ppm, fw; ±SD) and mean trophic levels (if available) for penguin populations (n = 26) from five distinct marine ecosystems in the Southern Hemisphere. SPSLME = Southern Patagonian Shelf Large Marine Ecosystem. (See Supp. Table 1 for trophic level calculations.)

Common name	Species	Marine ecosystem	Population	n[a]	Feather Hg (ppm)	Trophic level	Source (Hg; $\delta^{15}N$)[b]
Rockhopper	*Eudyptes chrysocome*	SPSLME	Staten Island	61	5.10 ± 1.46	3.16	This study; A. Raya Rey, unpublished data
		Southern Indian Ocean	Kerguelen Islands	12	1.96 ± 1.41	3.91	Carravieri et al. (2013)
Adélie	*Pygoscelis adeliae*	Southern Ocean	Antarctic Peninsula	21	0.35 ± 0.09	3.80	This study
		Southern Ocean	South Georgia	3	1.40		dos Santos et al. (2006)
		East Antarctica	Syowa Station	10	0.17 ± 0.4		Honda et al. (1986)
		East Antarctica	Syowa Station	10	0.09 ± 0.05		Yamamoto et al. (1996)
		Ross Sea	Terra Nova Bay	3	0.82 ± 0.13	3.71	Bargagli et al. (1998) and Cherel et al. (2008)
Chinstrap	*P. antarctica*	Southern Ocean	Antarctic Peninsula	16	0.62 ± 0.30	3.80	This study
Gentoo	*P. papua*	Southern Ocean	Antarctic Peninsula	21	0.31 ± 0.10	3.90	This study
		Southern Ocean	South Georgia	20	0.85 ± 0.88	4.21	This study; Stowasser et al. (2012)
		Southern Ocean	South Georgia	14	0.95 ± 0.85	4.21	Becker et al. (2002) and Stowasser et al. (2012)
		Southern Ocean	South Georgia	2	0.54	4.21	dos Santos et al. (2006)
		Southern Indian Ocean	Kerguelen Islands (Estacade population)	12	5.85 ± 3.00		Carravieri et al. (2013)
		Southern Indian Ocean	Kergueln Islands (Penn Island population)	12	1.44 ± 0.44		Carravieri et al. (2013)
Macaroni	*Eudyptes chrysolophus*	Southern Indian Ocean	Kerguelen Islands	12	2.24 ± 0.29	3.91	Carravieri et al. (2013)
		Southern Ocean	South Georgia	20	3.41 ± 0.73		Becker et al. (2002)
African	*Sphenicus demersus*	Benguela upwelling ecosystem	South Africa	19	1.00 ± 0.44	3.21	This study; Barquete (2012)
Little	*Eudyptula minor*	Bass Strait	Phillip Island	19	2.00 ± 0.77	4.38	This study; Chiaradia et al. (2014)
		Bass Strait	St. Kilda	18	5.01 ± 1.78	5.35	S. Caarels, unpublished data; Kowalczyk et al. (2013)
Magellanic	*S. magellanicus*	SPSLME	Staten Island	18	2.91 ± 0.56	4.50	This study; A. Raya Rey, unpublished data
		SPSLME	Isla Martillo	16	1.79 ± 0.34	5.32	This study
		SPSLME	Punta Tombo	21	0.21 ± 0.10	5.56	Frías et al. (2012) and Forero et al. (2002)
King	*Aptenodytes patagonicus*	Southern Indian Ocean	Kerguelen Islands	12	2.22 ± 0.59	4.18	Carravieri et al. (2013)
		Southern Indian Ocean	Crozet Islands	31	1.98 ± 0.73	4.35	Scheifler et al. (2005) and Cherel et al. (2007)
		Southern Ocean	South Georgia	20	3.01 ± 0.79	4.80	This study
Emperor	*A. fosteri*	Ross Sea	Terra Nova Bay	3	0.98 ± 0.21	4.76	Bargagli et al. (1998) and Cherel et al. (2008)

[a] Sample size for Hg concentration.
[b] Only one citation indicates both Hg and $\delta^{15}N$ from same source.

species and sex as factors as well as the interaction between them. Inter-annual variation in mercury concentrations in Southern Rockhopper penguins was also examined as samples were collected over the course of three breeding seasons. A one-way univariate ANOVA was used to examine the effect of year on mercury concentration in this species. Tukey's HSD were used for post hoc comparisons in all ANOVAs. All means (mercury and $\delta^{15}N$) are reported ± SD and statistical significance was defined at $p < 0.05$.

3. Results

3.1. Mercury exposure and trophic dynamics in penguins

To date, the Estacade breeding population of Gentoo penguins in the Kerguelen Islands shows the highest feather mercury concentrations (5.85 ± 3.00 ppm) reported for the Order Sphenisciformes. Two other populations, Little penguins from St. Kilda (5.01 ± 1.78 ppm) and Southern Rockhopper penguins (5.10 ± 1.46 ppm) from Staten Island also showed elevated mercury concentrations. For Southern Rockhopper penguins, there was a significant effect of year ($F_{2,61} = 16.0$, $p < 0.001$) in which mercury concentrations in 2012/2013 were significantly lower than in both 2009/2010 and 2011/2012 (Tukey's HSD, $p < 0.001$ in both cases). Macaroni and King penguins from South Georgia had mercury concentrations over 3.00 ppm, while 18 out of 26 populations examined had mercury concentrations ⩽2.00 pm (Table 1, Fig. 2). Within this latter group, *Pygoscelis* and Emperor penguin populations in the Southern Ocean and Magellanic penguins from Punta Tombo had the lowest mercury exposure (<1.00 ppm). No effect of sex was detected among species for which these data were available.

Using both literature and field collected data we compared penguin populations for which both adult feather mercury concentrations and stable isotope data ($\delta^{15}N_{penguin}$ and $\delta^{15}N_{primary\ consumer}$) were available (Table 1, Fig. 3). These included populations from breeding sites in the Southern Indian Ocean (Kerguelen Islands and Crozet Island), the Southern Ocean (Antarctic Peninsula, South Georgia, and the Ross Sea), the Southern Patagonian Shelf Large Marine Ecosystem (Staten Island, Isla Martillo, and Punta Tombo), and Bass Strait in Coastal Australia (Phillip Island and St. Kilda). For the majority of penguin populations investigated, foraging at a high trophic level was associated with elevated mercury exposure (Table 1, Fig. 3). Even so, no correlation was found between mean population mercury concentration and trophic level for the 20 penguin populations analyzed here ($r = 0.079$, $p = 0.741$). Several penguin populations, however, deviated from the overall trend of increasing mercury concentration with increasing trophic level. Southern Rockhopper penguins from Staten Island had one of the highest feather mercury concentrations, but foraged at one of the lowest trophic levels recorded (Figs. 3 and 4). Conversely, the Magellanic penguin population at Isla Martillo had a relatively high trophic level, though mercury concentrations were near the average for all species (Figs. 3 and 4). In the Antarctic Peninsula, all three *Pygoscelis* penguins fed within 0.10 trophic levels of each other; however, Chinstrap penguins had higher mercury concentrations than Adélie and sympatric Gentoo penguins.

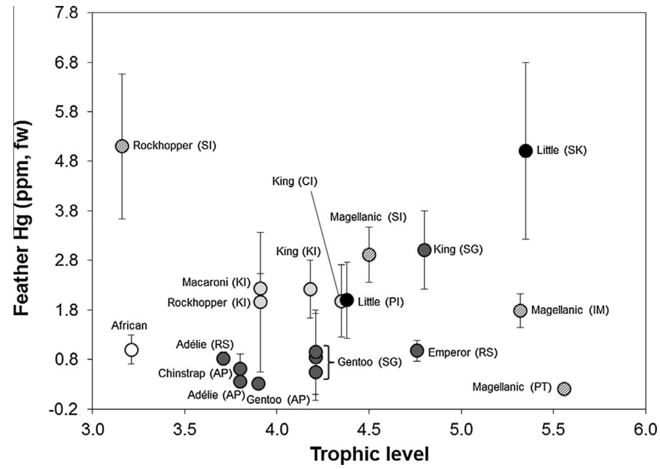

Fig. 3. Average adult feather mercury concentrations (±SD) and calculated trophic levels of penguin populations in Southern Hemisphere marine ecosystems: Southern Ocean (dark grey), Benguela upwelling ecosystem (white), Bass Strait (black), the southern Patagonian large marine shelf ecosystem (hashed), and Southern Indian Ocean (light grey). Population specified as follows: AP = Antarctic Peninsula, SG = South Georgia, RS = Ross Sea, KI = Kerguelen Islands, CI = Crozet Islands, IM = Isla Martillo, SI = Staten Island, PT = Punta Tombo, PI = Phillip Island, SK = St. Kilda.

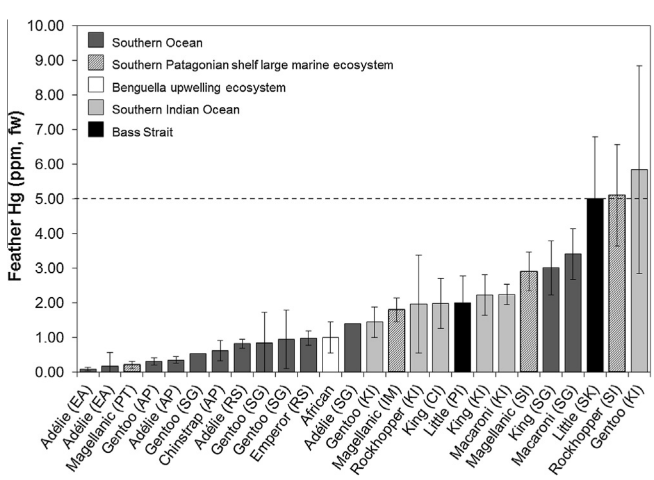

Fig. 2. Mercury concentrations in adult body feathers from penguin populations in five distinct marine ecosystems: Southern Ocean, Southern Indian Ocean, Southern Patagonian shelf large marine ecosystem, Benguela upwelling ecosystem, and Bass Strait. For species with multiple populations within/among ecosystems: AP = Antarctic Peninsula, SG = South Georgia, EA = East Antarctica, RS = Ross Sea, KI = Kerguelen Islands, CI = Crozet Islands, IM = Isla Martillo, SI = Staten Island, PI = Phillip Island, and SK = St. Kilda. Dashed line represents lowest observable adverse effects level in seabirds (Wolfe et al., 1998; Burger and Gochfeld, 2000; Evers et al., 2008). Sample sizes can be found in Table 1, error bars ± SD.

3.2. Geographic variation in mercury exposure and trophic levels among penguin populations

Mercury concentrations in King penguins from the Kerguelen Islands were significantly lower than in those at South Georgia ($t = 3.22$, df = 28.4, $p = 0.003$), a likely result of foraging at a slightly lower trophic level or limited bioavailability of mercury at the former location (Table 1). There was no difference in mercury exposure between King penguin populations at the Kerguelen and Crozet Islands; which coincided with both populations foraging at similar trophic levels. Mercury concentrations were higher in the King penguins at South Georgia relative to the Crozet Islands ($t = -3.54$, df = 19.5, $p = 0.002$) with the latter population foraging nearly half a trophic level higher. Mercury concentrations and trophic levels were available for four Gentoo penguin populations, three of which were from South Georgia. Gentoo penguins in the Antarctic Peninsula had a lower mean mercury concentration than populations in South Georgia ($p < 0.02$ in both cases, with the exception of the population from dos Santos et al., 2006 which did not provide a standard deviation to allow for statistical comparison) and fed 0.31 trophic levels lower.

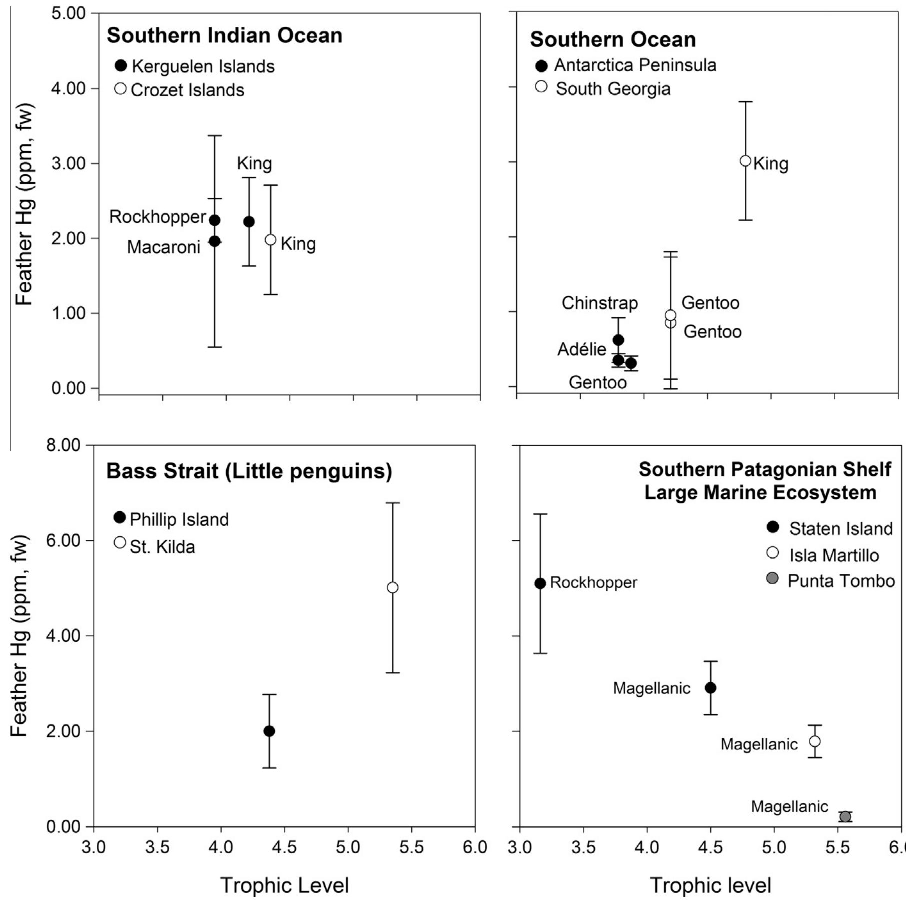

Fig. 4. Relationships between adult feather mercury concentrations and calculated trophic level varied among marine ecosystems. A limited relationship between mercury concentration and trophic level was detected among penguin populations in the Southern Indian Ocean. Positive relationships between mercury concentration and trophic level were detected among penguin populations within the Southern Ocean and Bass Strait marine ecosystems. An inverse relationship between mercury concentration and trophic level was detected among penguin populations in the Southern Patagonian Shelf Large Marine Ecosystem.

Southern Rockhopper penguins at Staten Island had significantly higher mercury concentrations than those at the Kerguelen Islands ($t = -7.01$, df = 16.0, $p < 0.0001$) despite foraging 0.43 trophic levels lower. Among Magellanic penguin populations, birds at Staten Island had significantly higher mercury concentrations than those at Punta Tombo ($t = 20.18$, df = 17.9, $p < 0.0001$) and Isla Martillo ($t = 22.52$, df = 30.2, $p < 0.0001$). Although foraging a full trophic level higher than the population at Staten Island and at a slightly higher trophic level than the population at Isla Martillo, the Magellanic penguins at Punta Tombo had the lowest mercury concentrations in this species (Table 1, Fig. 4). Further, the Magellanic penguin population at Punta Tombo had nearly the lowest mercury concentration among all penguin populations (Fig. 2). A significant difference in mercury exposure was detected between Little penguin populations at Phillip Island and St. Kilda ($t = 6.61$, df = 22.9, $p < 0.0001$) in which the mean mercury concentration at St. Kilda averaged 3.01 ppm higher than in the Phillip Island population (Table 1). Little penguins from St. Kilda fed at nearly a full tropic level higher than the population at Phillip Island (Table 1, Fig. 4).

4. Discussion

While trophic level certainly provided explanatory power for differences in mercury among some penguin populations, there was no overall correlation between population-level mercury concentration and trophic level overall as a result of several populations foraging at lower trophic levels than expected given their high mercury concentrations. Three populations had mercury concentrations that exceed documented lowest adverse effect levels in adult feathers (5.0–40.0 ppm; Wolfe et al., 1998; Burger and Gochfeld, 2000; Evers et al., 2008): Southern Rockhopper penguins at Staten Island, Little penguins at St. Kilda, and the Estacade population of Gentoo penguins from the Kerguelen Islands (Carravieri et al., 2013). Little penguins at St. Kilda foraged at a higher trophic level than their congeners at Philip Island, providing explanatory power to the elevated mercury concentrations. Alternatively, Southern Rockhopper penguins at Staten Island foraged at the lowest trophic level of all populations despite a mixed diet of juvenile and larval fish, crustaceans, and cephalopods (Raya Rey and Schiavini, 2005). Our results suggest that in some cases local environmental factors impacting the bioavailability of mercury were the most important drivers of mercury exposure rather than trophic level alone.

4.1. Mercury exposure and trophic relationships among Southern Ocean penguin populations

Chinstrap penguins in the Antarctic Peninsula had higher mercury concentrations than sympatrically breeding Adélie and Gentoo penguins despite foraging at a similar trophic level (Fig. 4). This inter-specific difference is consistent among breeding colonies of *Pygoscelis* penguins throughout four of the major regions of the Antarctic Peninsula (Brasso et al., 2012). Higher mercury concentrations in Chinstrap penguins likely result from this species' preference for foraging on mesopelagic lantern fish (*Electrona antarctica*; Ainley, 2002; Brasso et al., 2012). Fish and seabirds that forage on mesopelagic prey or at mesopelagic depths (100–1000 m) show an enhanced bioaccumulation of mercury relative to their epipelagic, trophically similar congeners (Monteiro et al., 1996; Monteiro and Furness, 1997, Choy et al., 2012, Ferriss and Essington, 2011). Adélie and Gentoo penguins preferentially feed on epipelagic, yet trophically similar, species such as the Antarctic silverfish (*Pleuragramma antarcticum*; Ainley, 2002; Pinkerton et al., 2013). Thus, the higher mercury concentrations in Chinstrap penguins relative to their congeners may be explained by enhanced bioavailability of mercury to their mesopelagic prey to which Adélie and Gentoo penguins are not often exposed.

Inter-specific differences in mercury exposure at South Georgia were readily explained by trophic level differences. King penguins at South Georgia had significantly higher mercury concentrations than sympatric Gentoo penguins and foraged at a higher trophic level (Table 1, Fig. 4). The diet of Gentoo penguins at South Georgia is dominated by krill (*Euphausia superba*) with fish (Family: Nototheniidae) comprising <30% of the diet (Croxall et al., 1997). The diet of the King penguin is comprised of 97% fish (Family: Myctophidae; Olsson and North, 1997) supporting the trophic level and mercury exposure differences detected here. In addition, the mesopelagic foraging habitat of myctophid fishes as well as King penguins themselves provides further explanatory power to the elevated mercury exposure in this population (Monteiro et al., 1996; Monteiro and Furness, 1997, Choy et al., 2012). Overall, differences in trophic level or foraging habitat provided the most explanatory power for inter-specific differences in mercury exposure in the Southern Ocean (Fig. 4; see also Brasso and Polito, 2013).

4.2. Intra-specific comparisons of mercury exposure and trophic level in Adélie, King, and Gentoo penguins

Owing to their wide geographic distribution in the Southern Ocean, we were able to conduct intra-specific comparisons among geographically distinct populations of Adélie, King, and Gentoo penguins. In general, Adélie penguins had the lowest mercury concentrations across all species. Mercury concentrations in East Antarctic Adélie penguin populations were the lowest and were highest at South Georgia; however, stable isotope data was not available from these populations for comparison with the Antarctic Peninsula and Ross Sea. Though the mercury concentration in the Ross Sea population was significantly higher than the population in the Antarctic Peninsula ($t = -6.0581$, df = 2.28, $p = 0.02$), these populations appear to forage at similar trophic levels (Table 1). Brasso and Polito (2013) found mercury concentrations in chick down from Adélie penguins in the Ross Sea to also be significantly higher than in the Antarctic Peninsula; however, this was due to a mirrored difference in trophic level between populations. For King and Gentoo penguins, significant population-level variation in mercury uptake among populations of both species appears to be driven by differences in trophic level. King penguins presented a clear relationship with diet in which mercury concentrations were highest in the population (South Georgia) that foraged at a higher trophic level (Table 1). Higher mercury concentrations in Gentoo penguins from South Georgia relative to the Antarctic Peninsula were also explained by a slightly elevated trophic level in the former population relative to the latter (Fig. 4). However, it is important to note that the small differences in trophic level detected among populations do not eliminate the possibility that mercury may be more bioavailable to penguins at South Georgia relative to other regions.

For Gentoo penguins, differences in foraging habitat may be as important as trophic level as a major driver of variation in mercury exposure among populations. Throughout their range Gentoo penguins display significant plasticity in dietary composition (proportion of krill vs. fish) and foraging behavior (inshore/offshore, benthic/pelagic) at both the individual and population levels relative to their congeners (Cherel and Hobson, 2007; Miller et al., 2009; Carravieri et al., 2013). For example, Carravieri et al. (2013) found a significant, positive relationship between mercury concentrations and stable isotopes (δ^{15}N, δ^{13}C) in Gentoo penguins at the Kerguelen Islands reflecting a succession of inter-individual foraging preferences from birds feeding exclusively on crustaceans within an enclosed bay to demersal fish in the open ocean. Mercury concentrations in the sub-population of Gentoo penguins at the Kerguelen Islands feeding within an enclosed sea (Penn Island), primarily on pelagic crustaceans, had an average mercury concentration almost 4.50 ppm lower than a nearby population foraging on a greater proportion of fish in open water (Fig. 3; Estacade population, Carravieri et al., 2013). Trophic level was not calculated for Gentoo penguins at the Kerguelen Islands for inclusion in the present study as baseline δ^{15}N values, thought to vary between the foraging locations (pers. comm., A. Carravieri and Y. Cherel), have not been published. The relatively high degree of inter-individual and population-level variation in mercury concentrations and foraging habits among Gentoo penguin populations reaffirms the importance of assessing risk of mercury exposure at the population, rather species level in marine ecosystems.

4.3. Mercury concentrations in African penguins

Together with an earlier report (Brasso et al., 2013) our studies have provided the only documented mercury concentrations for wild African penguins and for the Benguela upwelling ecosystem as a whole. With breeding colonies in close proximity to anthropogenic activity and subsequently potential point sources of mercury emissions, it was surprising that African penguins had relatively low exposure to mercury. Ultimately, the relatively low trophic level of African penguins aligned with the low feather mercury concentrations suggesting limited bioavailable mercury in this marine ecosystem. Documenting mercury exposure in African penguins was of critical importance due to their dramatic population decline over the past century; the IUCN status of this species was recently changed to 'Endangered' (Crawford et al., 2011; BirdLife International, 2013; Trathan et al., 2014). Though changes in diet from biomass fluctuations in the anchovy fishery have previously been identified as a major cause of population decline (Crawford and Dyer, 1999; Crawford et al., 2011), low mercury concentrations documented herein rule out mercury toxicity as an accompanying cause of reproductive failure or adult mortality. Our findings support the notion that in the Benguella upwelling ecosystem, the most worrisome form of marine pollution may be limited to effects of oil pollution and increased sedimentation from coastal industries (Trathan et al., 2014). Ultimately, with a similar diet to other seabirds in the Benguela upwelling ecosystem (foraging primarily sardines *Sardinops sagax* and anchovies *Engraulis encrasicolus*; Crawford and Dyer, 1999; Crawford et al., 2011), African penguins should continue to be used as a biomonitor of mercury and other marine pollutants in this ecosystem.

4.4. Mercury 'hot spot' for Little penguins in Port Phillip Bay

While previous studies have reported mercury concentrations from muscle, liver, and kidney of Little penguins (see Gibbs, 1995; Choong et al., 2007) this is the first study to document feather mercury concentrations in this species—a tissue more readily comparable with other seabird biomonitoring efforts. Differences in dietary preferences provided explanatory power for variations in mercury exposure detected between the populations of Little penguins from St. Kilda and Phillip Island (Table 1, Fig. 4). With breeding colonies located only 70 km apart, the foraging ranges of birds in these populations overlap in Port Phillip Bay between the pre-egg laying and incubation periods (Chiaradia et al., 2012). Though dietary composition is similar between populations, the proportion of each prey type consumed varies in that the population at St. Kilda consumes primarily anchovy (*Engraulis australis*) with a relatively smaller proportion of squid (*Loliolus noctiluca*; Chiaradia et al., 2012). The diet of the Phillip Island population is more diverse, comprised of krill (*Nyctiphanes australis*), Barracouta (*Thyrsites atun*), and arrow squid (*Nototodarus gouldi*) as well as a smaller proportion of anchovy relative to the St. Kilda population (Chiaradia et al., 2012). In addition, penguins from the St. Kilda population preferentially prey upon a smaller size class and potentially from a different stock of anchovy relative to the Phillip Island population, further separating the foraging niche between populations (Chiaradia et al., 2012). Ultimately, a high trophic diet dominated by anchovy is the likely cause of the elevated trophic level and subsequently elevated mercury concentrations in the St. Kilda population relative to the population at Phillip Island.

Despite clear evidence of trophic level disparities as the mechanism driving the difference in mercury exposure between populations of Little penguins, it is important to also note the differences in the year-round foraging areas of these two populations. While the penguins from Phillip Island forage in open-water, coastal areas during summer, the St. Kilda population feeds within Port Phillip Bay year-round, a densely populated, shallow enclosed bay subject to dredging from channel deepening projects and a high volume of marine traffic. Thus, we considered that Little penguins from St. Kilda may be exposed to point sources of mercury in this heavily industrialized bay in addition to foraging at an elevated trophic level compounding the risk of exposure to mercury. However, in a geographic analysis of heavy metal concentrations (including mercury) in fish within six distinct fishing zones along the Victorian coast, Fabris et al. (2006) did not find heavy metal concentrations within Port Phillip Bay to be higher than in surrounding coastal fishing zones. Recent changes in environmental management practices have reduced historically elevated mercury concentrations (Fabris et al., 2006) suggesting enhanced bioavailability of mercury in this bay may be more limited than we initially predicted.

4.5. Mercury 'hot spot' for Southern Rockhopper and Magellanic penguins at Staten Island

Southern Rockhopper and Magellanic penguins at Staten Island have the highest feather mercury concentrations ever reported for these species despite occupying lower trophic levels relative to their conspecifics (Figs. 3 and 4). The Magellanic penguins breeding on Isla Martillo, within the Beagle Channel approximately 170 km southwest of Staten Island, foraged at a higher trophic level, yet had lower mercury concentrations than the population at Staten Island. Further, the Magellanic penguin population at Punta Tombo foraged at the highest trophic level, but had the lowest mercury concentrations suggesting that environmental factors, rather than trophic differences may be driving variation in mercury exposure throughout this species' range. A similar pattern was found among Southern Rockhopper penguin populations. Southern Rockhopper penguins at the Kerguelen Islands foraged at a higher trophic level than Southern Rockhopper penguins at Staten Island, but mercury concentrations averaged $\sim$3.00 ppm higher in the latter population. By ruling out trophic level as a primary factor explaining high mercury concentrations in penguin populations at Staten Island, our findings suggest enhanced bioavailability of mercury to penguins and their prey as the result of an environmental 'hot spot' of mercury availability in the vicinity of the Le Maire Strait and coastal areas surrounding Staten Island. Local nutrient loading, bacterial and/or planktonic community structure, bathymetry, and deep sea sediment composition are all important factors driving the bioavailability of mercury in marine ecosystems (Cossa et al., 2011; Mason et al., 2012); however, the factors affecting the biogeochemical cycling of mercury have been largely overlooked in this region of the South Atlantic. It is currently unclear what factors, in the absence of a documented point source of mercury, may be contributing to the high bioavailability of mercury to penguins breeding in the vicinity of Staten Island relative to a nearby population in the Beagle Channel and Magellanic penguins to the north at Punta Tombo.

The mercury concentrations detected herein are of particular concern for Southern Rockhopper penguins at Staten Island as they exceeded published lowest observable adverse effects levels in seabirds (5.0–40.0 ppm, Wolfe et al., 1998; Burger and Gochfeld, 2000; Evers et al., 2008). Now listed as 'vulnerable' (BirdLife International, 2012) owing to striking population declines throughout its breeding range (Hilton et al., 2006), the population at Staten Island was considered stable to increasing until 1998 (Schiavini, 2000). However, new assessments have documented another decline in this large breeding population (Raya Rey et al., 2014)—Staten Island is home to approximately 14% of the global population of Southern Rockhopper penguins (Birdlife International, 2012). Thus, elevated mercury concentrations in one of the largest breeding populations of Southern Rockhopper penguins in South America are of immediate concern as the global decline of this species would increase significantly if this population suffered adverse effects from mercury toxicity.

5. Conclusions

To our knowledge, the present study is the most comprehensive population-level assessment of mercury concentrations among seabird populations in the Southern Hemisphere. The wide geographic distribution of penguins within this hemisphere provided a unique opportunity to use members of a single taxonomic group (Sphenisciformes) as biomonitors of mercury among a variety of geographically distinct marine ecosystems. We have highlighted the utility of trophic level calculations in ecotoxicological studies by identifying penguin populations at 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. To create this comprehensive data set, mercury concentrations and $\delta^{15}N$ values from field collected samples and literature derived data span a $\sim$17 year time period. Several studies have documented significant inter-annual variation in diet and mercury uptake (Gariboldi et al., 2000; Frederick et al., 2002; Brasso and Cristol, 2008; Rigét et al., 2011), a factor we were not able to control for in the present study. Therefore, it is also possible that inter-annual variation in diet and mercury concentrations may provide additional explanatory power to differences in mercury concentrations detected among populations.

Of the 26 penguin populations considered here, the Southern Rockhopper penguins at Staten Island, Little penguins at St. Kilda, and the Estacade population of Gentoo penguins at the Kerguelen Islands warrant further research to determine if mercury is impacting reproduction and/or chick survival and to identify specific environmental sources of mercury. Population-level responses to elevated mercury concentrations have previously been documented as a result of decreased reproductive effort or poor breeding success in Arctic and Antarctic seabirds (Tartu et al., 2013; Goutte et al., 2014). Short of reductions in mercury emissions and careful monitoring of point sources of pollution, there is little that can be done to prevent mercury accumulation by wildlife, whether in a terrestrial, aquatic, or marine ecosystem. However, detailed descriptions of the biogeochemical and geothermal processes responsible for creating the mercury 'hot spots' identified herein would offer much needed insight into understanding pathways of exposure in these understudied marine ecosystems. It is worth noting that compared to mercury concentrations reported in other seabird populations, penguins are at the lower end of exposure in marine ecosystems. Mean feather mercury concentrations in 10 species of procellarid seabirds from South Georgia ranged from 2.69 to 27.43 ppm with the highest concentrations found in Northern Giant Petrels (*Macronectes halli*) and Wandering Albatross (*Diomedea exulans*; Anderson et al., 2009).

Despite a lower risk of exposure to harmful concentrations of mercury, we suggest that penguins can serve as valuable biomonitors of mercury availability in marine ecosystems throughout the Southern Hemisphere. Foraging at moderate to high trophic levels and nesting in large, readily accessible colonies facilitates the use of penguins as model species and as a proxy for other taxa that are more difficult to sample including marine mammals or species that are more sensitive to human disturbance (e.g., petrels and albatross; deVilliers et al., 2006). The wide geographic distribution of penguins allows for the use of taxa from within a single family limiting the need to consider evolutionary or physiological variation in the ability to regulate or eliminate mercury. Further, the unique, annual catastrophic molt cycle of penguins drastically diminishes intra-individual variation in body feather mercury (Brasso et al., 2013)—an issue common in Procellariform seabirds owing to their protracted molt periods (Bridge, 2006). As sensitive biomonitors of local changes in mercury availability in marine ecosystems, penguins can provide succinct information regarding population-level exposure to allow the identification of potential mercury 'hot spots' throughout the Southern Hemisphere.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.marpolbul.2015.05.059.

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